TECHNICAL REPORT
Number: 001-92

THE BEHAVIORAL TOXICOLOGY OF HIGH-PEAK, LOW AVERAGE POWER, PULSED MICROWAVE IRRADIATION

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25 January 1993

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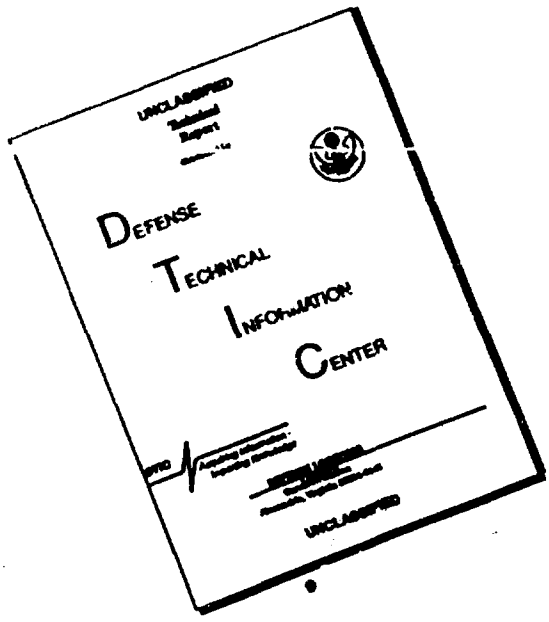

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	25 January 1993	Interim; 20 Oct 89 - 31 Dec 92

4. TITLE AND SUBTITLE
The Behavioral Toxicology of High-Peak, Low Average Power, Pulsed Microwave Irradiation.

5. FUNDING NUMBERS
PE: 62787A
PR: 3M162787A878
TA: BB
WU: 042

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Microwave Research
Division of Neuropsychiatry
Walter Reed Army Institute of Research
Washington, DC 20307-5100

8. PERFORMING ORGANIZATION REPORT NUMBER
001-92

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research & Development Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
APPROVED FOR PUBLIC RELEASE:
DISTRIBUTION UNLIMITED

13. ABSTRACT (Maximum 200 words)

The TEMPO microwave bioeffects exposure platform at the Department of Microwave Research, Walter Reed Army Institute of Research, produces an 80 ns, 3 GHz microwave pulse with a peak power of 700 MW. The energy transmitted is approximately 16 J, so that at a pulse repetition rate of 0.125 Hz, the duty factor is 10^{-8} and the average power is 2 W. The time-averaged SAR for TEMPO exposures at full power is 0.072 W/kg, a value well-below suggested exposure limits. Five behavioral tasks were used to determine if exposure affected subsequent task performance: temporal bisection, Y-maze, treadmill running, food motivation (behavioral economics), and Persolt swim test. Reliable effects were found with the temporal bisection, Y-maze, treadmill, and behavioral economics tasks. The pattern of results suggests at least two effects of high-power pulsed microwaves: memory impairment and decreased physical endurance. It was suggested that research be expanded in this area, and that tasks which are specifically sensitive to memory function and physical endurance receive especial attention.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Microwave Bioeffects, Motivation, High Power Microwave, Behavior, Rats, time perception, time discrimination, Decision-making, memory, endurance, depression	96
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED

THE BEHAVIORAL TOXICOLOGY OF HIGH-PEAK, LOW-AVERAGE POWER, PULSED MICROWAVE IRRADIATION

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ABSTRACT

The TEMPO microwave bioeffects exposure platform at the Department of Microwave Research, Walter Reed Army Institute of Research, produces an 80 ns, 3 GHz microwave pulse with a peak power of 700 MW. The energy transmitted is approximately 16 J, so that at a pulse repetition rate of 0.125 Hz, the duty factor is 10^{-8} and the average power is 2 W. The time-averaged SAR for TEMPO exposures at full power is 0.072 W/kg, a value well-below suggested exposure limits. Five behavioral tasks were used to determine if exposure affected subsequent task performance: temporal bisection, Y-maze, treadmill running, food motivation (behavioral economics), and Porsolt swim test. Reliable effects were found with the temporal bisection, Y-maze, treadmill, and behavioral economics tasks. The pattern of results suggests at least two effects of high-power pulsed microwaves: memory impairment and decreased physical endurance. It was suggested that research be expanded in this area, and that tasks which are specifically sensitive to memory function and physical endurance receive special attention.

FOREWORD

This paper is one of a series of occasional, informal accounts of work in the Department of Microwave Research (DMR) at the Walter Reed Army Institute of Research. The reports generally address topics in the safety of electromagnetic radiation and are intended to inform Department of Defense audiences with an interest in such issues. Although their contents may overlap partly with our publications in the scientific literature, most papers are based on in-house research and are intended to be more accessible to a wide audience of readers with various technical backgrounds. The materials are the author's personal views and not the official positions of the Army or the WRAIR. Comments to the senior author are welcome.

This work was supported by Research Area III, Health Hazards of Military Systems, of the U.S. Army Medical Research and Development Command; MG Travis, Commanding.

The research described in this report was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to the principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 85-23.

The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4-3, AR 360-5).

PREFACE

THE PURPOSE OF WRAIR'S RADIOFREQUENCY BIOLOGY PROGRAM

The historic focus of WRAIR's radiofrequency biology program has been to perform basic research on personnel hazards associated with non-ionizing radiation in military settings. The formulation of specific goals has been guided by the technical assessments of the Army systems development community, and so a long-term close collaboration has existed with relevant Army Materiel Command communities.

The chapters presented in this report focus on biological effects produced by one high peak power pulsed microwave source operating at a center frequency of 3 Ghz. With this source and another HPPM source working at 1300 MHz a large number of conditions in the operating parameter space of interest to the Army has been explored. As the technical details of directed energy technologies become better defined with the evolution of deployable directed energy systems, the biology research is adjusted to become increasingly relevant. A convergence of generic research and specific technical requirements is occurring. Even though the experimental data reported in this publication were derived from a laboratory HPPM source, the relevance to weapons systems under consideration is fairly high.

The purpose of this research is to confer to the systems development community a carefully and rigorously derived data base from which assessments on safety and integrity of task performance can be made. Seldom does the information make a clean separation between hazards and non-hazards and interpretation is often necessary. Extrapolations from animal data are always in question. Whether an "effect" represents a hazard or non-hazard is frequently intricate. The biomedical community cannot merely hand over a self-contained data base to the engineering community without continuing dialogue between the two communities as systems are integrated into the force structure.

The information yield in this report is illustrative. The observation that very high peak - but low average - power, which would be considered permissible by some safety standards, produces a degradation of performance in animals leads immediately to the issue of extrapolation to humans. If the extrapolation can be made, do we need to protect from such effects? Are the effects tolerable? Are they tolerable in some situations and not others? To address these and

other questions more fully, we need a more encompassing picture of an individual in a military directed energy environment. As that picture emerges piecemeal the biomedical community must remain engaged in the tech development process.

The biomedical data base must be continually refined through a vigorous research program. Scientists must be willing to abandon doctrinaire, preconceived notions as new information comes to light. There is a controversy in the scientific community as to the reality of so-called non-thermal effects, such as mentioned above. Vigorous debate and alternate approaches should be encouraged, although Project Reliance may have dealt such a process a major blow.

Lastly, the latest revision of the ANSI standard for non-ionizing radiation has taken some cognizance of the possible hazards of peak power by imposing a 100 kV/m peak on narrow RF pulses. The work cited in this report used peaks in excess of 100 kV/m. This has reinforced the conviction of staff scientists at Walter Reed that "lower" high peak pulses at faster repetition rates, such that the average energy would allow such exposures under the new rules, should be studied. In all cases constant technical guidance from the weapons development community is needed to maintain maximum relevance to the needs of the Army.

ACKNOWLEDGEMENT

Research in the area of bioeffects subsequent to electromagnetic radiation is highly interdisciplinary, as evidenced by the list of authors of this report. However, many other individuals also contributed to and ensured the success of the experiments detailed below. The principal investigators take special pride in acknowledging the real reason for their success.

Engineering support in the form of maintenance and operation of TEMPO was provided by Mike Belt. He was often assisted by Dave Varle in this crucial job. Without them, none of the experiments would have been performed. Bill York also provided crucial support to this project in numerous ways. Environmental control of the anechoic chamber for TEMPO and of the housing of the equipment was his primary responsibility and headache. Experimental sessions had perfect temperature and humidity for machine, man, and animal through his efforts. Mr. York also was responsible for the fabrication of the animal restraints which were used during exposures. Rob Serafini and Michelle DeAngelis were responsible for animal care and handling, and performed data collection in several of the experiments. Their excellent attention to detail and efficiency in scheduling substantially contributed to the projects. Finally, Chris Klein handled many thorny administrative and management problems associated with this project. His efforts are a fine example of what effective management can actually accomplish.

High quality research can only be accomplished by high quality people performing at their highest level of effort. The quality and quantity of the research reported here are a testament to the efforts of all of the team at the Department of Microwave Research.

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"The only way to keep your health is to eat what you don't want, drink what you don't like, and do what you'd rather not."

Mark Twain

"A memorandum is written not to inform the reader but to protect the writer."

Dean Acheson

SECTION 1: INTRODUCTION

Current microwave occupational safety standards (ANSI, 1992; NRCP, 1986) are primarily designed to avoid tissue damage through heating. As a result, they do not address the issue of the maximum peak power allowed during exposure to high-peak power pulsed microwave (HPPM) fields. While current standards suggest that whole-body specific absorption rate (SAR) exposures be limited to 0.4 W/kg and/or a local SAR of 8 W/kg, there is no limit on the peak power that can be used to achieve these doses. For instance, all the time-averaged whole-body exposures in Table 1 would be within the suggested safety limit even though the peak SARs vary over several orders of magnitude.

Table 1: Constancy of Time-averaged SAR For Various Values of Peak SAR

Time-averaged SAR (W/kg)	Peak SAR (W/kg)	Exposure Duration (s)	Specific Absorption (J/kg)
0.4	0.4	360	144
0.4	4.8	30	144
0.4	1.44×10^{3}	10^{-2}	144
0.4	1.44×10^{7}	10^{-6}	144
0.4	1.44×10^{11}	10^{-10}	144
0.4	1.44×10^{15}	10^{-14}	144

To date, research on the behavioral effects of microwaves has focused on low power, continuous wave (CW) microwave radiation which places little or no thermal burden on the organism (see reviews by Adey, 1981; Gage, 1984; Michaelson, 1986). Fewer studies have employed pulsed microwaves (Bermant, Reeves, Levinson, and Justesen, 1979; Carroll, Levinson, Justesen, and Clarke, 1980; D'Andrea, Cobb, and deLorge, 1989; deLorge, 1979a, b; deLorge and Ezell, 1980; Frey, Feld, and Frey, 1975; Hjeresen, Doctor, and Skeldon, 1979; Hunt, King, and Phillips, 1975; Johnson, Meyers, Guy, Lovely, and Galambos, 1976; King, Justesen, and Clarke,

1971; Lebovitz, 1983; Roberti, Heebels, Hendricx, deGreef, and Wolthuis, 1975; Schrot, Thomas, and Banvard, 1980; Thomas, Burch, and Yeandle, 1979; Thomas, Finch, Fulk, and Burch, 1975; Thomas, Schrot, and Banvard, 1980, 1982). The power density in these studies is quite low, often because the pulse repetition rates are very high (up to 1 kHz). An even smaller number of these studies have compared pulsed and CW radiation at the same average power density (Cain and Rissman, 1978; Frey, Feld, and Frey, 1975; Kholodov, 1975; Lebovitz, 1983; Roberti, Heebels, Hendricx, deGreef, and Wolthuis, 1975; Thomas, Finch, Fulk, and Burch, 1975; Thomas, Schort, and Banvard, 1982). Five of the seven studies above found a greater effect on behavior with pulsed microwave radiation. Although this suggests that pulsed microwaves interact differently with biological systems than CW, it must be emphasized that none of these studies used high levels of peak power in their exposures.

The experiments, in this report, are focused on the safety of pulsed, high power microwaves which may be encountered by personnel during military operations. The military use of directed energy, for instance, results in the production of radiofrequency fields of very high peak power density for very short periods of time. Under these conditions, the average power density to which personnel is exposed is quite low, although the peak power density is enormous. To illustrate, suppose that a microwave generator operating at 3 GHz is producing 40 ns pulses at 500 MW with a 0.2 Hz repetition rate (one pulse/5 s). In the far field only a fraction of that power impinges on an absorbing surface. Suppose the power density at some point in the far field is 0.8 mW/cm^2 per Watt of radiated power. Then at an output of 500 MW the power density exposure of personnel at that point would be 400 x 10^6 mW/cm^2. Since this power density is only realized for 40 ns periods, the average power density (measured over a standard six min period) would, of course, be lower than the exposure limit of 10 mW/cm^2 which is recommended by ANSI (Polk and Postow, 1982). For example, if we sample power every 40 nanoseconds, there would be 72 periods with power at 400 x 10^6 mW/cm^2 and 8.99 x 10^9 periods with power at 0 mW/cm^2 in a 6 min period. This averages out to 3.2 mW/cm^2 for the 6 min measurement period. By contrast, the typical peak power density in the studies cited above is on the order of 1 x 10^2 W/cm^2 with a typical average power density of 10 mW/cm^2.

Thus there is reasonable concern that adverse effects may result from extremely short-duration microwave exposures of extremely high peak-power. The question, then, is "Can a very brief, high power pulse of energy cause an injury?". At the time that the series of experiments reported here was initiated, there were very few behavioral studies which addressed that question.

As noted above, there was some indication that low-power pulsed microwave radiation has greater behavioral effects than equivalent (power density) CW radiation. Subsequently researchers at the USAF School of Aerospace Medicine (Cordts, Merritt, Erwin, Hardy, and Yochmowitz, 1988; Klauenberg, Merritt, and Erwin, 1988) began to explore peak power densities in the kW/cm^2 range.

Cordts et al. (1988) exposed rats to a Cober peak power simulator operating at 2.066 GHz with a 250 ms pulse width and a Gypsy Vircator operating at 1.64 GHz with a 140 ns pulse width. For the Cober source the maximum reported power density was approximately 30 W/cm^2, while for the Gypsy source the maximum power density was approximately 8 kW/cm^2. Three behavioral tasks, one-trial avoidance (of microwaves), drinking, and rotarod performance, were used to evaluate the effects of these two types of exposures. Only the drinking test (time spent drinking) was reliably affected by either source, but similar effects were seen for both sources despite the large difference in incident power density. Cordts et al. (1988) argue that the reduction in drinking time which was observed in both groups was, potentially, caused by different mechanisms. They estimated that a 0.7 °C increase in body temperature was caused by the Cober exposure, while the Gypsy exposure caused only a 0.0084 °C increase. This suggests that the effect of the Gypsy exposure was not thermal, but was due to some other, unspecified, direct effect of the exposure.

Klauenberg et al. (1988) exposed rats to a TEMPO vircator operating at 1.26 GHz with a 85 ns pulse width and a power density of 0.5 kW/cm^2. Startle responses (following single and multiple microwave pulses) and rotarod performance were used to behaviorally assess the effects of exposure to this system. Reliably more animals in the exposure group startled relative to the sham-exposure group when single pulses were used, but no differences between groups occurred during multiple pulse exposures. More exposed animals failed to complete the rotarod task than sham-exposed animals, but this difference was not reliable.

Both of these studies suggest that high-power, pulsed microwaves may be effective in altering behavior, but neither study adequately documents a relationship between an absorbed dose of power and a change in behavior. Specific absorption rates (SARs) were calculated rather than measured, and power density was highly variable (58% uncertainty noted by Klauenberg et al., 1988). While both studies are commendable for their groundbreaking efforts, it is clear that much remains to be done.

For instance, none of the experiments described above examined the effects of HPPM on cognitive function or sensory information processing. The series of experiments described below examines the effect of high-peak, but low-average, power microwave fields on a variety of behavioral tasks in rats. These tasks are designed to provide the knowledgeable practitioner of behavioral psychology with information concerning a variety of psychological processes in animal subjects, including sensation, perception, cognition (memory and decision-making), motivation, mood, and physical endurance. Because of the interdisciplinary nature of this field, reports of this work in the scientific literature may not adequately communicate with specialists outside of psychology. A major objective of the present report is to communicate our findings, many of which can be found in the scientific literature, to those non-psychologists who may find them relevant to their own work.

The approach which was utilized in examining the effects of HPPM exposure is often used in behavioral toxicology: subjects were given a non-lethal acute exposure to the suspected toxicant and were subsequently tested for an effect. This, however, is not the only possible approach. For this reason the reader should bear in mind that every method for studying a problem has both advantages and limitations. We will highlight the limitations of our choice to aid the reader's critical evaluation of our results.

Because of the difficulty of instrumenting experiments in a HPPM field, all of our experiments studied post-exposure effects of HPPM radiation. The lack of an effect in this type of design does not preclude effects which occur prior to or after the observation period. Thus there may be behavioral effects during the exposure, or more immediately after the exposure that could not be measured by our methods. Moreover, in most instances, the observations we made were only a "snap-shot" of the behavior in time. The duration of behavioral effects (or lack thereof) are not addressed by experiments which make only one observation following exposure.

The decision to use behavior as an end-point was dictated by the training of two of the principal investigators (TGR and YA) and by the fact that, in this field, behavior is widely believed to be the most sensitive indicant available (Gage, 1984). We again caution the reader, that methods often determine (in part) results. In other areas (for instance, behavioral pharmacology) it is as well-established that behavior can be extremely insensitive to manipulations which have profound effects on other measures. The lack of behavioral effects does not preclude physiological or toxicological effects of HPPM, and a behavioral effect does

not guarantee a lesion in the central nervous system that can be detected with current methods. At present, the correlation of behavior with anatomy and physiology is far from perfect.

With the exception of the bisection experiment, all exposures were acute rather than chronic. Questions concerning cumulative effects, as with the duration of effects, must await future research in this area. Due to time and equipment limitations, certain questions which naturally flow from an examination of the trade-off between dose, dose-rate and exposure duration have also not been examined.

To facilitate the readability of this report, the experiments, which each utilize similar microwave exposure methods but different behavioral methods, are described individually following a description of the microwave exposure system (TEMPO) and the methods that were used to determine the SAR of the exposures. The descriptions of the behavioral experiments can be read (or skipped) in any order without (we hope) detriment to the understanding of the material. Background information and references concerning the behavioral methods used are provided for the interested reader.

SECTION 2: METHODS AND MATERIALS

2.1 The TEMPO Exposure System

All exposures took place in a Keene Corporation Ray Proof anechoic chamber (16 ft. by 16 ft. by 12 ft.) at WRAIR's Department of Microwave Research laboratories. The TEMPO transmitter produces an 80 ns, 3000 MHz RF pulse. The transmitted power varies, a characteristic inherent in virtual cathode oscillators, from < 100 to ≈700 MW, within the 80 ns pulse duration. In terms of energy transmitted (≈16 J), the irregular TEMPO pulse may be represented as a rectangular pulse of 200 MW for 80 ns (see Figure 1). At a repetition rate of 0.125 Hz, the duty factor is 10^{-8} and the average power is 2 W.

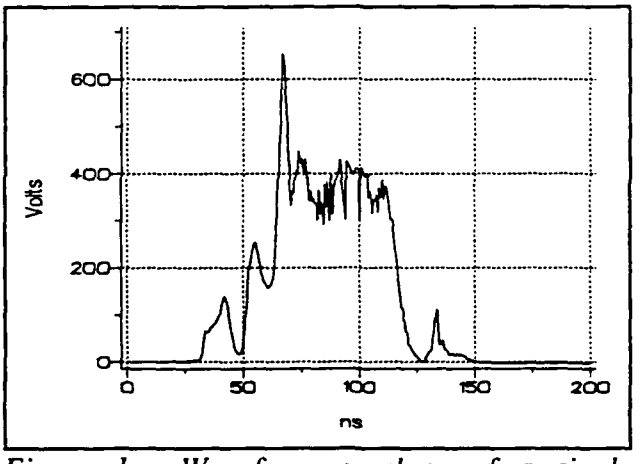

Figure 1. Waveform envelope of a single TEMPO pulse.

The TEMPO antenna is a longitudinally slotted circular waveguide which radiates a fan-shaped (narrow horizontal, wide vertical) horizontally polarized beam. Subjects are exposed in a dual corner reflector assembly, 2.25 m from the antenna, that allows simultaneous exposure of two animals. The corner reflectors focus the radiated energy and provide a 10 dB enhancement of the field with respect to the free-field power density of 0.1 mW/cm^2 per watt transmitted. Thus, at maximum transmitted power (700 MW), TEMPO achieves a power density of 700 kW/cm^2 and a field strength of 1.5 MV/m. Each animal was restrained in a cylindrical plastic enclosure and positioned on an RF transparent platform in the reflector.

Wire screens of varying mesh densities were placed over the antenna aperture to attenuate the output power of the system. Relative to the unattenuated output of the system, these screens produced power outputs of -20, -30, -40 and -50 dB.

In addition to microwaves, TEMPO produces soft X-rays. Ionizing radiation exposures for each condition are presented in Table 2. With the exception of the -50 dB power condition, the doses are fairly homogeneous across power levels. All doses are well below the level at which any behavioral sequela have been observed in rats (Fields, 1957; Mele, Franz, and Harrisson, 1988, 1990).

Table 2: Ionizing Radiation Dose (mREM ± SD) For Each Microwave Power Attenuation Condition

0 dB	-20 dB	-30 dB	-40 dB	-50 dB	Sham	Cage
281.25 ± 12.60	295.71 ± 32.50	307.14 ± 7.14	288.57 ± 57.21	47.5 ± 30.92	*	*

* Not measurable

Sound pressure levels (SPLs) for each exposure condition are presented in Table 3. The SPL for the full power (0 dB attenuation) condition was 65.69 dB, and the sound levels for all other power levels were approximately 20 dB higher. The peak SPL was approximately 88 dB with a majority of the acoustic energy in the frequency range between 2 and 6 kHz. In quiet conditions (sham exposures) the ambient SPL in the chamber was 56.55 dB. There are no known long-term psychological effects in rats from exposure to noise with these characteristics (Cure and Rolinat, 1992; Lai, 1987, 1988; Lai, Carina, Horita, and Guy, 1989).

Table 3: Noise Levels (dBA) for Each Microwave Power Attenuation Condition

0 dB	-20 dB	-30 dB	-40 dB	-50 dB	SHAM	CAGE
65.69	88.64	88.00	88.64	88.21	56.55	65.00

2.2 Dosimetry

The specific absorption rate (SAR) may be used to describe the rate of energy deposition within the entire biological specimen or at specific locations in the subject. In support of this series of experiments, calorimetric and thermometric techniques were used to evaluate both whole-body and spatially localized SARs in cadavers.

For both calorimetry and thermometry, a 1000 watt CW source was connected to the system in place of the TEMPO vircator. Whole-body SAR was determined using the single-well calorimetry techniques of Mathur, Akyel, and Lu (1992). Local SARs were determined using implantable, RF transparent Luxtron temperature probes. Euthanasia of the animals was accomplished by carbon dioxide inhalation.

2.2.1 Calorimetry Procedures

In behavioral experiments, animals are not anesthetized and are often minimally restrained to reduce stress. As a result, the animal does not stay in one position in the exposure area. For this reason, whole-body free-field exposures are performed, and, under such exposure conditions, knowledge of whole-body dose (SAR) is often thought to be more useful than localized dose in relating absorbed dose to the observed behavior. A detailed description of the calorimetry procedures can be found in Mathur et al. (1992).

The calorimetry performed for this series of experiments utilized a gradient layer Seebeck Envelope Calorimeter (SEC; Thermonetics Corp., Model SEC-A-1202, Serial Number 104, calibrated on 7/19/90). The SEC is used to determine the net heat load of the exposed animal. The SAR is then computed by dividing the net heat load by the duration of the exposure. In a SEC, the small temperature difference across the gradient layer is sensed by a multi-junction thermopile. The output of the thermopile is a direct measure of the heat flow rather than of temperature. The calorimeter output signal varies linearly with heat flow rate. The SEC principle has advantages over other calorimetry methods in that it is only necessary to measure heat flow rather than to contain or direct it. This avoids errors due to heat loss with variations in time and temperature.

Rat carcasses were put in a plastic bag and immersed in a water bath until the colonic temperature of the carcass reached 25 °C. This took 75 min on the average. The carcass was then transferred to a styrofoam box (to avoid heat loss) and was placed in the corner reflector for the exposure. Following the exposure, the carcass (in the box) was transported to the calorimeter and the carcass (without the box) was put into the calorimeter.

Data acquisition from the calorimeter was begun one hour prior to introduction of the rat carcass to determine a baseline value and was continued until the calorimeter output approximated the baseline value. This procedure took 3 to 4 h on the average. Sham-exposed carcasses were handled in a similar fashion except that they were not exposed to RF. Data acquisition software recorded the calorimeter output in volts.

2.2.2 Calorimetry Results

The results of the calorimetry indicated a whole-body SAR of 0.036 W/kg per watt transmitted. As noted above, the peak output of TEMPO is approximately 700 MW. Thus the peak SAR is 25.2 MW/kg. However, as noted above, the average output power of TEMPO is only 2 W. Thus the time-averaged whole-body SAR is 0.072 W/kg at full power. Exposures below full power would have appropriately adjusted SARs.

2.2.3 Thermometry Procedures

A more detailed description of the thermometry procedures can be found in Gambrill, DeAngelis, and Lu (1992) and in Lu, DeAngelis, and Gambrill (1992). Briefly, temperatures were monitored using a Luxtron Fluoroptic Optic Thermometric System (Model 3000 and MAM05 probes). Temperature data was recorded using a computer data acquisition system operating at 10 samples per second and was smoothed by a 1 Hz low pass filter. The raw temperature data was divided into pre-exposure, exposure, and post-exposure sections. For each section the relationship between temperature and time was determined using the linear curve fitting routine. The three sections were adjusted until the end points of the linear approximations intersected. The SAR was calculated using the information provided by the slopes of the three sections ($\Delta T/\Delta t$). Specifically, the SAR was then calculated as the exposure absorption rate less the average of the pre- and post-exposure rates.

2.2.4 Thermometry Results

Local SARs from CW exposures are presented in Figure 2. Twenty four different locations, including the brain, skin, and colon, were measured. The tip of the nose and the base of the tail had the highest SARs. SARs for the brain ranged from .057 to .087 W/kg per watt transmitted.

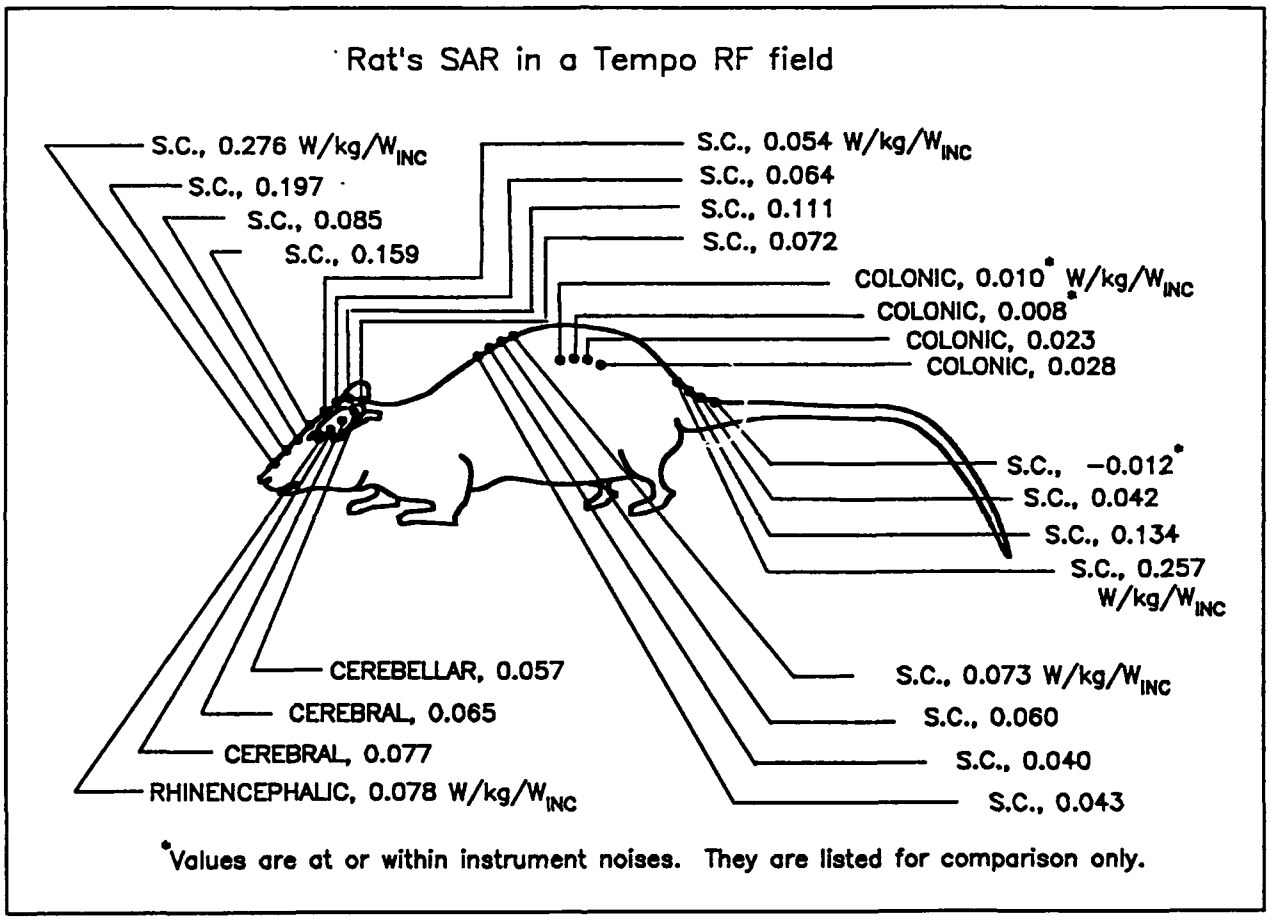

Figure 2. Thermometry results for TEMPO exposures in the rat.

SARs were determined using a CW source instead of the TEMPO source to avoid errors arising from the duration of TEMPO exposures (Stuchly and Stuchly, 1986). Keeping in mind that TEMPO exposures require approximately 25 min (see Section 2.3.1), we repeated our thermometry measurements following a TEMPO exposure to determine whether the very high peak-power emitted by TEMPO produces any measureable heating. The results of thermometry

conducted following 200 TEMPO pulses is shown in Table 4. The heating of the skin on the nose (which had the highest SARs following CW exposure) was only 0.41 °C, which is consistent with the calorimetry results.

Table 4: Temperature Changes Induced by 200 TEMPO Pulses	
Position of Sensor	**Temperature Changes (°C)**
Subcutaneous, 0.5 cm from nose tip	0.31
Subcutaneous, 1.0 cm form nose tip	0.41
Subcutaneous, 1.5 cm from nose tip	0.37
Subcutaneous, 2.0 cm from nose tip	0.32
Cerebrum	0.09
Anterior Cerebrum	0.09
Mid Cerebrum	-0.07
Rhinecephalon	-0.09

2.3 Subjects

Male, albino Sprague-Dawley rats, 90-110 days old at the start of each experiment served. All rats were allowed a short period of adaptation to the laboratory and individual housing. A 12 hr light: 12 hr dark cycle (lights on at 0600) was maintained throughout each experiment. Details concerning numbers of animals and special treatment (food and water deprivation) are detailed for each experiment.

2.4 Exposure Procedures

To maintain consistent exposure orientation and location within the microwave field, animals were placed into cylindrical plastic tubes during exposures. The tubes provided minimal restraint in that the animals could move legs and head but could not turn around. This ensured that the polarization of the animal in the field was maintained parallel with the E-field at all

times. Because even minimal restraint is capable of producing stress, the animals were adapted to the plastic tubes and placement in the anechoic chamber over a period of a week prior to actual exposures. Sham-exposed animals received similar treatment.

Two animals in separate restraining enclosures could be placed in the dual corner reflector at once, depending on the requirements of the experiment. Each exposure (with the exception of the motivation study, see below) consisted of 200 pulses at a pulse repetition rate of 0.125 Hz. The duration of sham exposures was approximately 25 min., which was the normal duration of actual exposures. During sham exposures the transmitter was not operated. Animals were immediately placed in the appropriate apparatus following exposures for behavioral testing. For cage control conditions, animals were simply taken from their home cages and placed directly into the apparatus for testing.

SECTION 3: TEMPORAL BISECTION [1]

As was noted above, previous experimental studies of HPPM effects on behavior have only scratched the surface with regard to the types of behaviors which might be affected by electromagnetic radiation. Since we are primarily interested in determining that HPPM does not affect human behavior, it is sensible and prudent to examine behaviors in animal subjects which have a functional similarity to human behaviors. By functional similarity it is meant that although the form of a task used with animals may not bear a physical or formal resemblance to the human task, the same psychological functions are required to perform the task. Thus, there are memory tasks for use with humans and memory tasks for use with animals, but there are, rarely if ever, tasks which appear the same. For example, a human test of memory would be verbal and might require reading instructions to the subject, following which the subject would be required to view a list of words and repeat those words (in order) after varying periods of time. An animal test of memory would not be verbal. It would involve training the animal to perform a task (instead of reading instructions), and the animal might be required to remember the location of a reward a short period of time after it was shown where it was hidden. Both tasks can be used to measure memory function, although they are very different in form.

In this section, we describe an experimental task which allowed us to examine several psychological functions in animals that are used everyday by humans. These included sensory information processing, memory, attention, and decision confidence.

3.1 Background

The simplest form of sensory information processing which can be studied is concerned with the relationship between physical stimulus magnitudes and perceived or psychological magnitudes (i.e., psychophysical scales). In humans, it is fairly easy to determine what the relationship is between physical stimulus magnitudes and perceived magnitudes: one can simply

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[1] *Portions of this work have been presented at the 63rd annual meetings of the Eastern Psychological Association in Boston, MA (Raslear and Akyel, 1992) and at the First World Congress for Electricity and Magnetism in Biology and Medicine in Lake Buena Vista, FL (Raslear, Akyel, Bates and Belt, 1992).*

ask how bright, how loud, etc., a physical stimulus is (Stevens, 1975). For humans, it is known that there is not a one-to-one relationship between perceived and physical magnitudes. For instance, equal changes in sound pressure are well-known not to result in equal changes in loudness. It is for this reason that the decibel scale was brought into use many years ago by audio engineers: equal changes in decibels more nearly approximate equal changes in the perception of loudness. A similar situation holds true for the perception of brightness, and can be easily demonstrated with the use of a standard 3-way light bulb which has equally spaced wattages[2]. The difference in brightness between the 50-watt and 75-watt outputs is much greater than the difference in brightness between the 75-watt and 100-watt outputs.

There are, currently, few behavioral techniques that can be used for determining psychophysical scales in animals (Raslear, 1991). Among these is the bisection technique which was first described by Boakes (1969) and subsequently employed by Raslear (1975, 1983, 1985) and Church's group (Church and Deluty, 1977; Meck, 1983; Maricq and Church, 1983; Maricq, Roberts, and Church, 1981). Recent theoretical and methodological advances (Raslear, 1982, 1983, 1985) have allowed the use of this method in behavioral toxicology (Raslear, Bauman, Hursh, Shurtleff, and Simmons, 1988) and behavioral pharmacology (Shurtleff, Raslear, Genovese, and Simmons, 1992) studies. Animal subjects can be exposed to various treatments or putative environmental hazards and be subsequently tested for changes in cognitive/perceptual function. The animal bisection method is ideally suited for this use because it has been demonstrated that the animal version of the task is the functional equivalent of the human task (Fagot and Stewart, 1970; Raslear, 1983; Raslear, Shurtleff, and Simmons, in press). The present experiment uses the temporal bisection technique to evaluate the perceptual and cognitive effects of exposure to high peak-power pulsed microwave irradiation.

3.2 Task Description

The bisection technique is designed to determine the psychological middle point of a sensory distance (bisection point or BP), and thus allows for the determination of equal sensory distances from which a psychophysical scale may be derived (see Raslear, 1982, 1983 for

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[2] *Strictly speaking the wattage of a light bulb is not a proper measure of light intensity, but it is close enough to use as an illustrative experiment that anyone can perform at home to see that our sensory systems actively transform and code the physical energy which impinges on us.*

details). For instance, if the BP is physically half-way between the physical stimuli, it is easy to see that perceived magnitude is identical with physical magnitude. However, the BP is usually not physically half-way (i.e., at the arithmetic mean) between the physical values of the two interval-defining stimuli. When this is the case, the functional relationship between perceived and physical magnitudes must be deduced.

When the bisection technique is employed with animals, some variant of the following procedure would be used. Initially, animals are trained to make one response (R_A) if a particular stimulus (S_A) is presented and a different response (R_B) if another stimulus (S_B) is presented. The two stimuli, S_A and S_B, define the interval to be bisected. When asymptotic performance has been reached (i.e., discriminative accuracy is consistently high), several stimuli with values intermediate to S_A and S_B (i.e., $S_A \leq S_x$, S_y, $S_z \leq S_B$) are interspersed with the training stimuli during sessions. In these "test" sessions correct responses to S_A and S_B are reinforced as before, but responses to the new stimuli are not (maintained generalization). A BP is determined by examining the relative frequency of one of the responses in the presence of each of the stimuli (the response chosen is irrelevant since there are only two responses and the functions will be complimentary). If, for instance, the relative frequency of R_A is chosen, the relative frequency of R_A will be near 1.0 for S_A, near 0 for S_B, and will have intermediate values for the intermediate stimuli (Figure 3). By interpolation, that stimulus value which would produce a 0.5 relative frequency of the designated response is determined and is defined as the bisection point (S_{AoB}) of the interval, S_A -S_B.

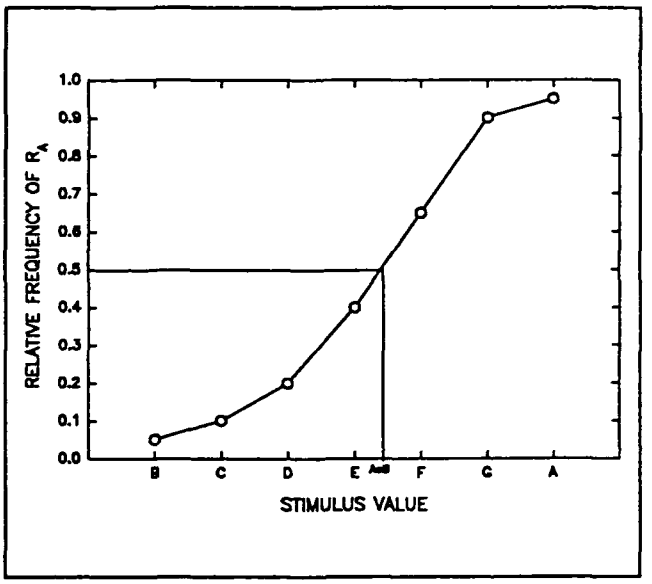

Figure 3. Psychometric function relating relative frequency of the response, "This is stimulus A", as a function of the stimulus value.

As previously described (Raslear et al., 1988; Shurtleff et al., 1990, 1992), performance on the bisection task is analyzed within the context of a simple, heuristic, information processing model of animal timing (Church, 1984; Gibbon and Church, 1984), as illustrated in Figure 4. This model contains a pacemaker or clock, which produces a mean rate of output (ticks/unit time) and an associated variance (variability in clock rate across observation periods). Clock variance can arise from a variety of sources either inherent in the pacemaker, or contributed by other functional elements of the model. Major sources of variance arise from memory and attention functions. The model has a memory function consisting of reference memory (relatively permanent memory) and working memory (short-term memory of a stimulus which has just been presented). Attention functions reside primarily in the switch which must open and close to allow accurate timing of a stimulus.

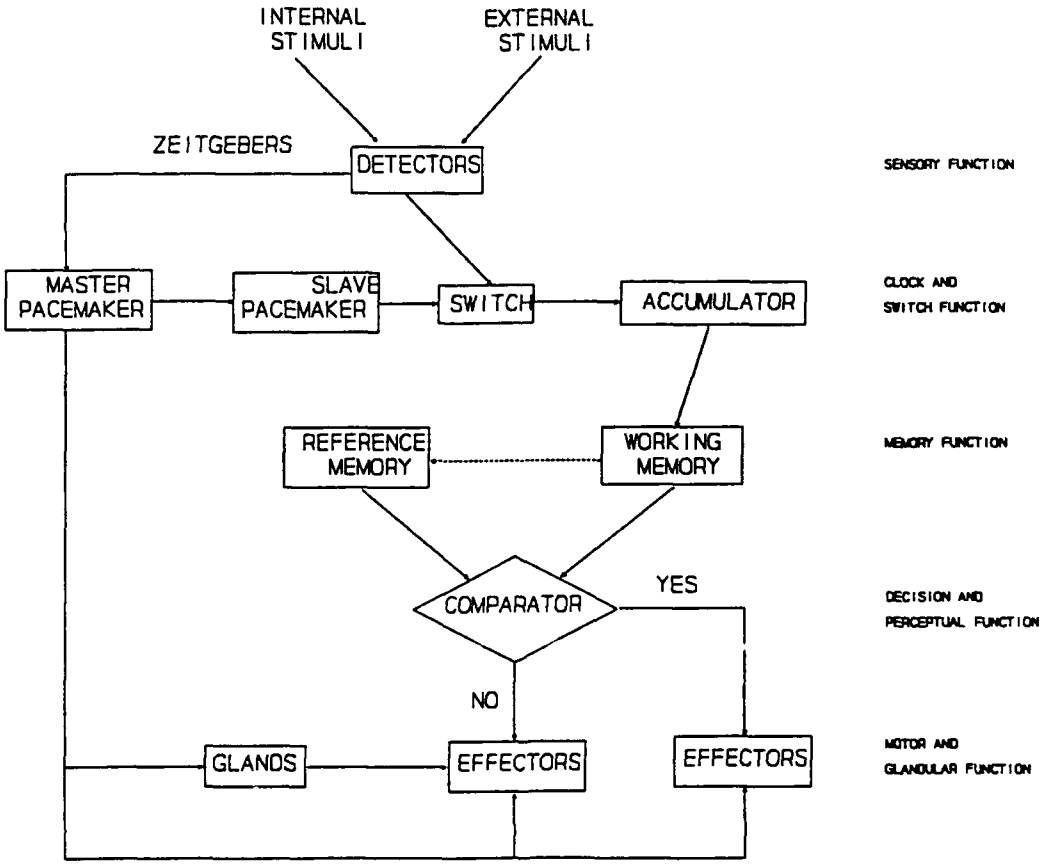

Figure 4. A heuristic model of the circadian and short duration event timing systems.

Timing in this model takes place in the following fashion. Within a variable period of time following the onset of a stimulus (say a light), the switch opens and the accumulator sums a number of clock ticks from the slave pacemaker. When the stimulus is offset, the switch closes, again with a variable latency. Since there is variance inherent in the slave pacemaker (due to its own variability and that of the master or circadian pacemaker), the same physical stimulus duration will not always produce the same sum of ticks in the accumulator. Moreover, opening and closing the switch is also variable and further increases the variability of the sum in the accumulator for any stimulus duration. The sum from the accumulator is sent to the working memory and it is then compared with the stimulus values which have been previously stored in the reference memory. Both the reference values and the working memory values are imprecise, and in the presence of noisy data, a statistical decision theory rule (e.g., maximize the per cent correct responses) is used to decide if the stimulus which was presented was stimulus A or stimulus B.

Our analysis provides two different measures of clock function. A' is a nonparametric signal detection theory measure of discriminability (Green and Swets, 1974) and depends upon both the mean clock rate and the variance of the clock (see Raslear et al., 1988). It is roughly equivalent to a percent correct measure, in that chance performance is indicated by a value of A' = 0.5 and perfect performance is indicated by a value of A' = 1.0. The BP is an estimate of the perceptual midpoint of the temporal interval under consideration and is a variance-free measure of clock function. Thus, the pattern of treatment effects on these two measures provides information concerning the functional locus of a treatment's effect on time perception. Treatments which affect the mean rate of the clock should produce a change in A' and BP, while treatments which affect the variance of the clock should only produce a change in A'.

Our analysis also provides two measures of the decision function. On each trial the animals make a decision concerning the duration of the stimulus which was presented (i.e., was it the "long" or the "short" stimulus?). The measure B" is a nonparametric signal detection measure of response bias (the tendency to over-report "long" or "short" stimuli). This measure provides an indication of the location of the subject's criterion for the decision to report "long" or "short" (remember that the information available for making a decision is noisy, so the animal sets a criterion to meet some decision rule. If the criterion is set low, most stimuli will be classified as "long", and if the criterion is set high, most stimuli will be classified as "short").

In addition, null responses (no response within the duration of a trial) were also recorded for each stimulus. Since it is well established that judgment time increases with decreasing confidence in the correctness of a judgment (Costermans, Lories, and Ansay, 1992; Guilford, 1954), null responses serve as an indicator of the animal's confidence in the decision.

Finally, session time was also measured and used as a general indicator of task performance. In drug studies, for instance, this measure is used to indicate that dose levels have been reached which are not pharmacologically of interest because the animal is too debilitated to adequately perform (Shurtleff et al., 1992).

3.3 Methods and Materials

3.3.1 Behavioral Methods

3.3.1.1 Subjects

Eight male, albino Sprague-Dawley rats, 90-110 days old at the start of the experiment served as subjects. Following a short period of adaptation to the laboratory and individual housing, the rats were reduced to 80% of their free-feeding weight. Water was freely available in the home cages and supplemental chow was provided, as needed, to maintain body weight. A 12 hr light: 12 hr dark cycle (lights on at 0600) was maintained throughout the experiment.

3.3.1.2 Apparatus

Two similarly constructed operant chambers (previously described by Raslear, 1983), which were located in a temperature- and humidity-controlled environmental chamber, were used. A PDP 11/73 computer was used to control the experiment and record data.

3.3.1.3 Procedures

General. The experimental session for each subject occurred at approximately the same time of day, 5 days per week excluding holidays. No water was available in the experimental chambers.

Discrimination training. A two-choice, discrete trial paradigm was used, in which the rats were trained to discriminate between two durations of light (1.12 W incandescent lamp mounted above the response levers in the operant chamber) that defined the temporal interval to be bisected (0.5 and 5.0 s). Responses were effective for 10 s following termination of the discriminative stimulus. A left or right lever response was reinforced with a single 45-mg Bio-Serv food pellet following termination of the 5.0 or 0.5 s stimulus, respectively. If no response was made during the 10 s interval the trial terminated and a null response was recorded. The intertrial interval was 10 s, during which time responses had no effect. Each stimulus occurred with equal probability on each trial.

A session consisted of 320 trials, of which the first 20 were warm-up, with 1.0 probability of reinforcement for correct responses. Performance on these trials was used to determine the condition the rat would experience for the next 300 trials. During discrimination training, if the animals produced a minimum of 90% correct responses during the warm-up, the remainder of the session consisted of non-corrected discrimination training in which the probability of reinforcement was set at a predetermined level. At the end of training and during the remainder of the experiment the probability of reinforcement was 0.25. Otherwise, a correction procedure was in effect in which an incorrect response to a discriminative stimulus resulted in that stimulus being presented again, 0.2 s following the incorrect response. The probability of reinforcement for a correct response was the same as in the non-correction procedure.

The probability of reinforcement for correct responses was initially 1.0 and was gradually reduced to .25 for individual animals over the course of 60 sessions. The criterion for changing reinforcement probability and initiating generalization testing was a minimum of 3 to 5 days under the noncorrection procedure.

Generalization testing. A maintained generalization procedure was used during the test phase. Five new durations, intermediate to the training stimuli, were presented in addition to the training stimuli. The five new stimuli were equally spaced on a logarithmic scale and were of the following durations: 0.74, 1.1, 1.62, 2.4 and 3.54 s. All seven stimuli were presented in random order throughout the session. Responses to the test stimuli were never reinforced, but responses to the training stimuli continued to be reinforced.

Testing sessions occurred on Tuesdays and Thursdays. A maximum of one exposure per week was scheduled for alternate Tuesdays and Thursdays. The remaining test session was used for a sham or cage control exposure. Discrimination training was conducted on the remaining three days of the week. The data from discrimination training were not used.

The animals were exposed to an ascending, then descending, series of microwave power outputs so that all power levels were tested twice.

3.3.1.4 Behavioral Data Analysis

The discriminability between training stimuli on generalization test days was computed for each rat using the nonparametric signal detection measure A' (Grier, 1971):

$$A' = \{[(HIT - FA) + (HIT - FA)^2] / (4 * HIT (1-FA))\} + 0.5,$$

where HIT refers to the probability of a response on the lever appropriate for the long stimulus (i.e., a "long" response) given the long stimulus was presented, and FA represents the probability of a response to the lever appropriate for a long stimulus given the short stimulus was presented. Values of this index can range from 0.5, an inability to discriminate between stimuli, to 1.0, perfect discrimination.

To estimate the BP, the psychometric function relating the proportion of "long" responses to the seven stimulus durations presented during generalization tests was constructed (Figure 3). The BP was determined by interpolation as the stimulus duration to which, during generalization testing, the rat was equally likely to respond long or short. This index is used to determine if the treatment altered the perceived duration of the stimuli presented. A longer BP duration would suggest stimuli were perceived as shorter than usual, and a shorter BP would indicate stimuli were perceived as longer than usual.

Response bias was indexed with the nonparametric signal detection measure B" (Grier, 1971):

$$B'' = [(HIT-HIT^2) - (FA-FA^2)]/(HIT-HIT^2+FA-FA^2).$$

This index can range from 1.0 (bias to report "short") to -1.0 (bias to report "long"). A value of B"=0 indicates an absence of bias.

3.4 Results

3.4.1 Psychometric Functions

Percent "long" responses were plotted as normal probabilities and stimulus durations were plotted with logarithmic spacing so that the psychometric function could be treated as a straight line (Guilford, 1954). Figure 5 presents the mean psychometric functions for the seven exposure conditions plotted in this fashion. Linear multiple regression analyses on the probability of "long" responses indicated that there was a reliable effect of log stimulus duration (b = 0.881, t = 20.96, df = 96, p < .00001), exposure condition (b= -.122, t= -3.69, df = 96, p = .0004), and the interaction of log stimulus duration and exposure condition (b= 0.109, t= 2.4, df = 96, p = .0185). There was no reliable effect of the order of exposure condition (ascending vs. descending).

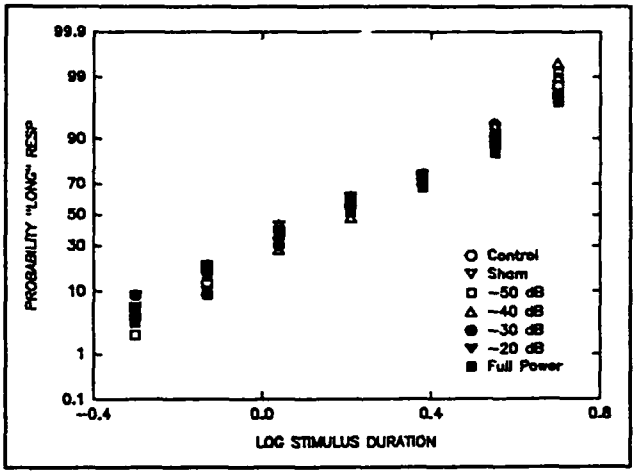

Figure 5. Probability of "long responses as a function of log stimulus duration and exposure condition.

The reliable exposure condition effects indicate that the slope of the psychometric function changed across conditions. This can be seen in Figure 5 as a steeper slope for the cage control, sham, -50 and -40 dB exposure conditions (open symbols) relative to the -30, -20 and 0 dB exposure conditions (closed symbols). Since the slope of the psychometric function is often used in classical psychophysics to characterize discriminability (Engen, 1971), this suggests that discriminability may have been decreased at the higher power levels.

3.4.2 Bisection Point

The reliable effect of exposure condition on the probability of "long" responses suggests two possible changes in the psychometric function other than slope: a change in the bisection point, and/or a change in response bias (an overall increase or decrease in the probability of "long" responses). Examination of Figure 5, however, does not appear to support either possibility.

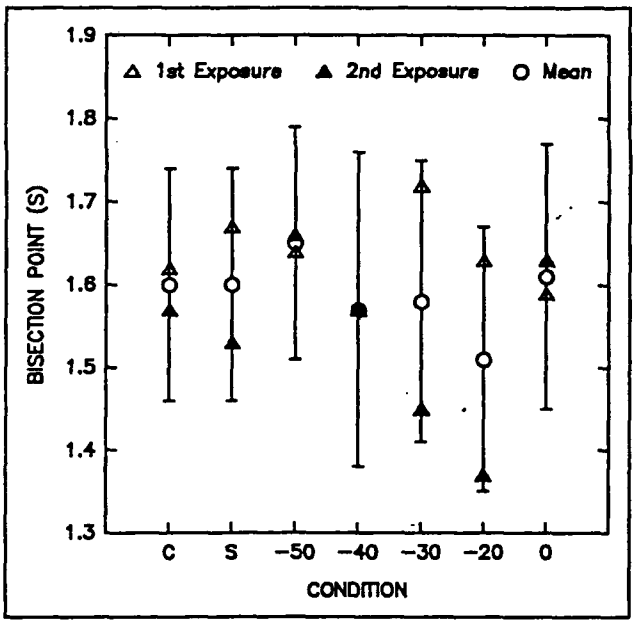

Figure 6. The bisection point as a function of exposure condition. The circles represent the mean of ascending (open triangles) and descending (filled triangles) determinations.

Bisection points for each exposure condition and order are presented in Figure 6. It is clear that exposure condition did not exert a systematic effect on the bisection points, a conclusion which is supported by an ANOVA ($F_{6,42} = 0.56$, $p > .05$). Similarly, there were no reliable effects of order ($F_{1,7} = 0.5$, $p > .05$) or of the interaction of order and condition ($F_{6,42} = 0.55$, $p > .05$).

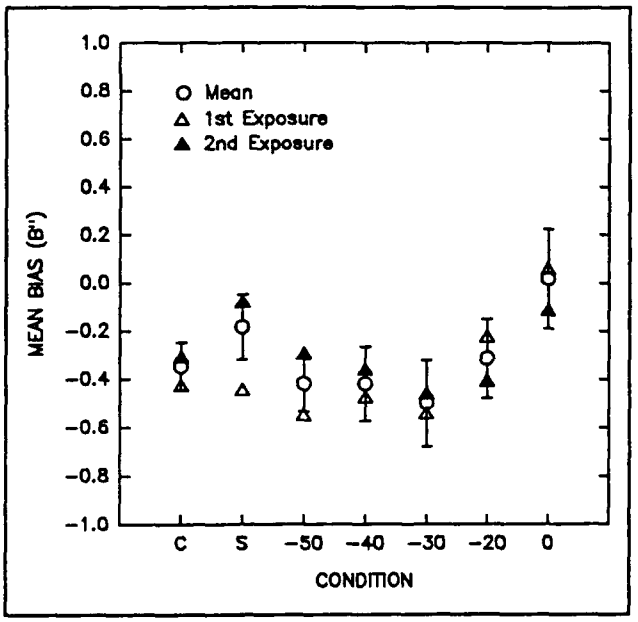

Figure 7. Response bias (B") as a function of exposure condition.

3.4.3 Response Bias

Overall, the rats tended to have a bias toward reporting "long", as can be seen in Figure 7 which presents the mean B" values for each exposure condition and order. However, exposure condition did not exert a systematic effect on response bias ($F_{6,42} = 1.26$, $p > .05$). There were no order effects ($F_{1,7} = 0.79$, $p > .05$) or interaction of order and condition ($F_{6,42} = 0.51$, $p > .05$).

3.4.4 Discriminability

As was noted above, a reliable change in slope in the psychometric function indicates that exposure condition affected discriminability. However, as can be seen in Figure 8, which presents the mean A' values for each exposure condition and order, a systematic change in discriminability with exposure condition is not evident. An ANOVA indicated a marginal effect of exposure condition ($F_{6,42}$ = 2.19, p = .063), but there was no effect of order ($F_{1,7}$ = 3.00, p > .05) or interaction of order and exposure condition ($F_{6,42}$ = 1.23, p > .05). A' in this instance provides only a point estimate of discriminability for the training stimuli (0.5 vs. 5.0 s).

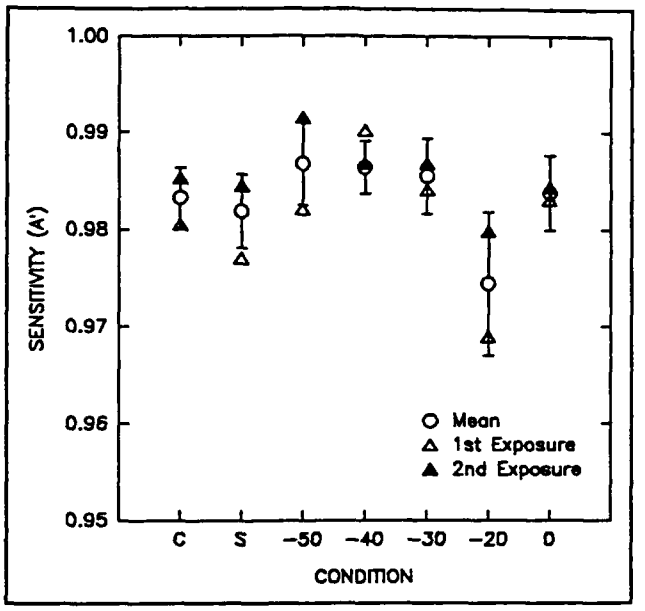

Figure 8. Discriminability or sensitivity (A') as a function of exposure condition.

3.4.5 General Task Performance

There are two measures of general task performance which are of considerable importance in behavioral studies of toxic substances and drugs. These are the total trials completed and the session time. In the present task, both total trials and session time are limited: 300 trials within 6300 s. Treatments which cause physical debility (somnolence, loss of motor control, etc.) can affect performance of the bisection task, with the result that all of the trials in a session are not completed. Often data from such sessions are erratic and are routinely discarded. Our previous research with drugs and the bisection procedure (Shurtleff et al., 1992) has indicated that rats fail to finish all 300 trials of a daily session at drug doses which are physically debilitating. In the present study, all rats completed 300 trials in every test condition, indicating that the exposures were not physically debilitating.

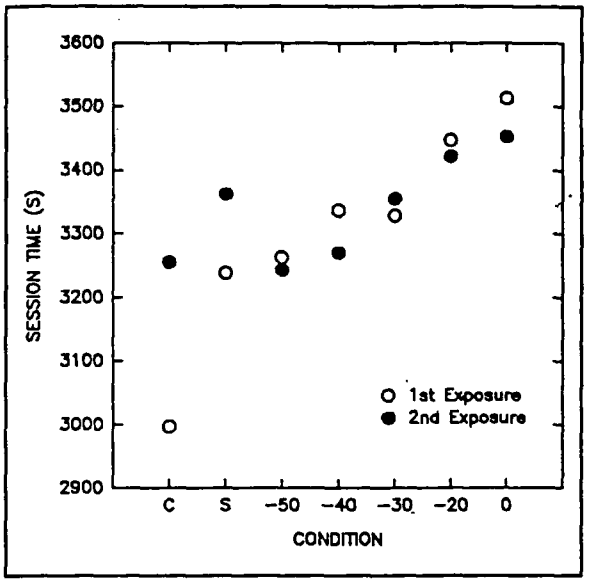

Figure 9. Session time as a function of exposure condition.

Examination of session time, however, reveals a definite increase in time to complete 300 trials as a function of exposure condition, as can be seen in Figure 9. This is a reliable effect ($F_{6,42} = 2.37$, $p < .05$), although there was no effect of order ($F_{1,7} = .02$, $p > .05$) or order by condition interaction ($F_{6,42} = 1.32$, $p > .05$).

3.4.6 Null Responses

Null responses occur when subjects take longer than 10 s to make a decision on a given trial. Given the increase in session time, it was expected that total null responses would increase as a function of exposure condition. Figure 10 shows that this did occur ($F_{6,42} = 2.62$, $p = .03$), but that there were no reliable order ($F_{1,7} = 0.51$, $p > .05$) or interaction ($F_{6,42} = 0.92$, $p > .05$) effects.

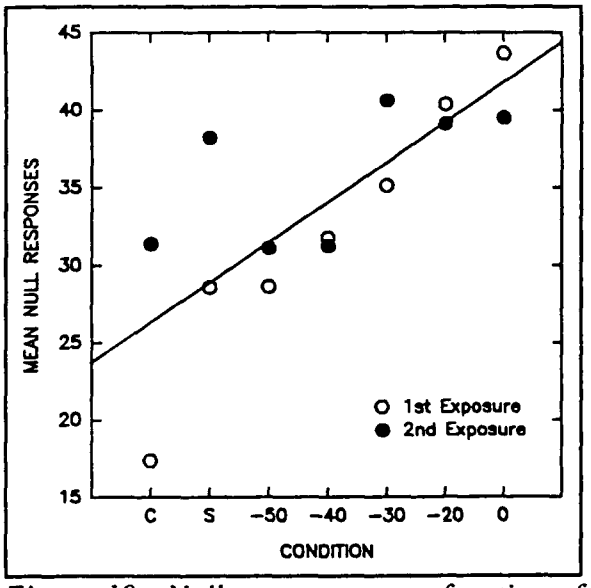

Figure 10. Null responses as a function of exposure condition.

3.4.6.1 Null Responses and Decision Confidence

The parallel increase in null responses and session time could reflect an increase in decision time, or a minor physical debilitation of the rats. In classical human psychophysics, a subject's confidence in a judgment or decision has an inverse relationship to the judgment time and to the magnitude of the stimulus differences being judged (Guilford, 1954; Pierrel and Murray, 1963). If null responses reflect confidence rather than physical performance, they should be inversely related to the subject's confidence in the judgment. Thus, the fewest null responses should occur at the training stimuli because the magnitude of the stimulus differences being

judged are the greatest. By the same logic, the highest number of null responses should occur at the bisection point.

Figure 11 shows the mean null responses for several exposure conditions as a function of stimulus duration. For every exposure condition, a similar pattern was observed: the fewest null responses occurred at the two training stimuli and the peak number of null responses occurred in the vicinity of the bisection point. This pattern was even observed in the cage control condition in which a change in behavior cannot be reasonably attributed to physical debility or some other performance factor. A multiple regression analysis of null responses as a function of log stimulus duration, squared log stimulus duration (to account for the quadratic effect evident in Figure 10), and exposure condition accounted for 87% of the variance in the data ($F_{3,45}$ = 110.75, $p < .00001$). Reliable effects of log stimulus duration ($t = 12.82$, $p < .00001$), squared log stimulus duration ($t = -17.63$, $p < .00001$), and exposure condition ($t = 3.78$, $p = .0005$) were found.

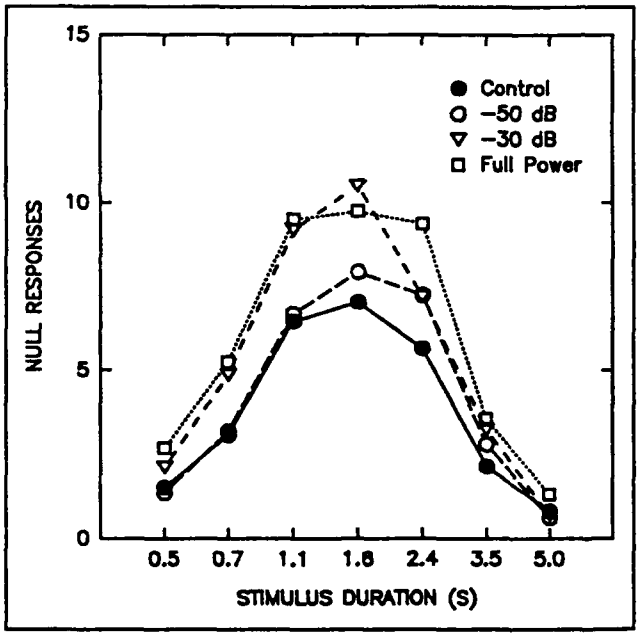

Figure 11. Null responses as a function of stimulus duration and exposure condition.

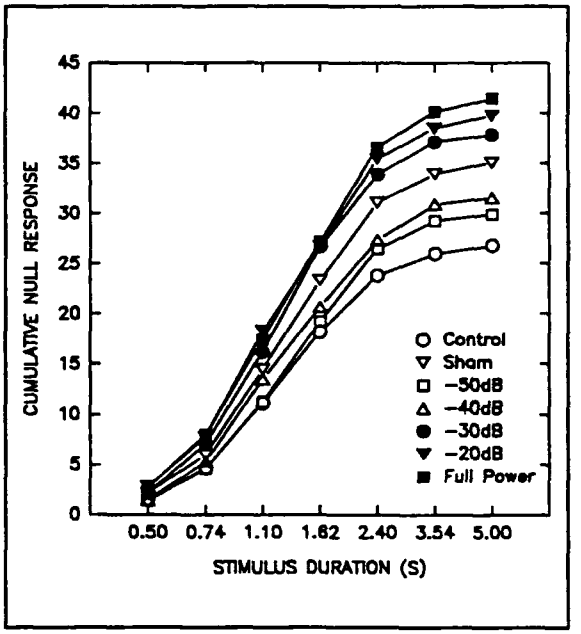

Figure 12. Cumulative null responses as a function of stimulus duration and exposure condition.

A putative lower limit for the effect of microwave exposure on decision confidence can be obtained by examining Figure 12. Figure 12 plots cumulative null responses (null responses summed successively across stimulus durations) for each exposure condition. In general, the full power condition has the highest cumulative null response function, the cage control condition has

the lowest cumulative null response function, and the remaining power levels are arrayed in order in between. The sham condition neatly divides the conditions into two groups between -40 and -30 dB. This suggests a time-averaged SAR in the range of 7.2 X 10^{-5} to 7.2 X 10^{-6} W/kg.

3.4.7 Ionizing Radiation and Acoustic Noise Effects

Table 5 presents the correlations between non-microwave exposures (X-ray and noise) and those behaviors for which there were reliable microwave effects. None of the correlations were reliable, which indicates that changes in the psychometric function (probability long), session time and null responses cannot be attributed to an effect of X-radiation or noise exposure.

Table 5: Correlation Coefficients (r), T-test statistics (t), and Probability Levels (p), For Covariations Between Noise and X-Ray Exposures and Those Behaviors Affected by Microwave Exposures.

NOISE			
	r	**t**	**p**
Probability Long	-.0646	-.222	0.6015
Session Time	.165	.227	0.8217
Null Responses	.126	-1.04	0.3031
X-RAY			
	r	**t**	**p**
Probability Long	-.0808	-.524	0.6015
Session Time	.183	.623	0.5362
Null Responses	.159	.421	0.6758

3.5 Discussion

The null response data clearly suggest that HPPM can affect cognitive function in rats within a two h period of time immediately following the exposure. A reliable dose-response function relating null responses and microwave power was demonstrated. This indicates that manipulation of microwave power produces orderly changes in null responses. Moreover, the lack of reliable exposure order (ascending vs. descending) or interaction effects between exposure order and power level demonstrates that this is a robust and replicable effect. Null responses measure the decision-making process of the rats because these responses are demonstrably under stimulus control. As expected from the human literature, null responses are a bitonic function of stimulus differences. Moreover, null responses retain a bitonic relationship to stimulus differences as they increase with microwave power. The threshold SAR for this effect could be as low as 7.2×10^{-5} W/kg. At full power the SAR was 0.072 W/kg, which is still considerably below the 4 W/kg SAR at which previous HPPM effects have been observed (D'Andrea and Cobb, 1987; D'Andrea et al., 1989) and about one-fifth of the current occupational safety standard.

The two measures of discriminability used in this study (slope of the psychometric function and A') are in apparent disagreement regarding the effects of HPPM. A' values did not change reliably as a function of power, but reliable slope changes did occur. The change in slope was noted between -40 dB and -30 dB, which is consistent with the results for null responses. The A' measure has the advantage of being bias-free in the sense of signal detection theory (Green and Swets, 1974), but provides information only concerning the discriminability of the training stimuli (a very easy discrimination). The slope of the psychometric function, on the other hand, relates information for all the stimuli presented, but is not bias-free. Bias, as measured by B" (again only for the training stimuli), however, also did not vary with power level. Thus, the difference between measures may be due to the greater sensitivity of the slope to HPPM effects because it assays more difficult discriminations which were not assayed by A'.

Previous studies of the effects of HPPM on timing behavior did not employ psychophysical tasks. Both D'Andrea and Cobb (1987) and D'Andrea et al. (1989) studied behavioral performance on a Fixed Interval (FI) schedule of reinforcement. While such performance is primarily under the control of time perception, it is well-known that other factors exert considerable control over FI performance (Gibbon, 1977). Thus, performance in

psychophysical tasks is not always consistent with FI performance (e.g., Shurtleff et al., 1990). Moreover, if the difficulty of the discrimination is crucial, as suggested above, it is unlikely that an effect would be detected in FI performance.

An effect on discriminability, in the demonstrated absence of an effect on the BP, suggests that HPPM is affecting the variance of the "internal clock" (Raslear et al., 1988; Shurtleff et al., 1990, 1992). Unfortunately, such variance arises from multiple sources, including memory, attention, the pacemaker, and the decision function. The results obtained for null responses suggests that cognitive functions may be particularly susceptible to HPPM effects. The data which will be presented in the next section of this report support that supposition and further suggest that changes in memory function may be responsible for the increase in null responses observed in the bisection task. Thus, we suggest that future research on HPPM effects focus more on cognitive tasks.

The ability to expose animals to very high peak-power, low average-power microwave fields within a controlled environment is almost unique. We have used that ability to directly address the issue of whether such fields can affect cognitive and perceptual function using behavioral methods which have been demonstrated (Fagot and Stewart, 1970; Raslear, 1983; Raslear et al., in press) to yield functionally equivalent information in animals and in humans. Thus, the data presented above constitute a unique contribution to the investigation of microwave effects on behavior.

SECTION 4: MEMORY CONSOLIDATION (Y-MAZE)[1]

In the previous section we observed that HPPM produces a dose-dependent increase in null responses and also a dose-dependent decrease in discriminability (slope of the psychometric function). Since the bisection point was not affected by HPPM, we concluded that the variance of the timing system was increased. This, in turn, indicates that one of several cognitive functions (memory, attention, decision function) could have been affected. Unfortunately, it is not possible to specify which cognitive function was affected with the bisection task. The Y-maze task, on the other hand, is specifically a test of memory consolidation. Thus, the results of this experiment can be used to suggest a more specific cognitive deficit than the bisection task.

4.1 Background

The primary objective of this experiment was to examine the effects of high peak, low average power, pulsed microwaves on memory function in rats. Current civilian and DOD safety regulations specify an exposure limit averaged over a six minute period (see Bassen, 1989; D'Andrea, Cobb, and de Lorge, 1989) but do not limit peak power. The exposures of interest in this experiment were of sufficiently short duration so as to not violate current safety regulations. However, the peak power levels are high enough to cause concern that they may have adverse effects, especially since there is a report that memory function in rats is affected by low-level, pulsed microwave exposure (Lai, Carino, Horita and Guy, 1989). Previous work in this area, as noted below, has not used the very high peak power levels which will be used in this protocol.

4.1.1 Memory Consolidation

It is common knowledge that a severe jolt to the human brain, caused by a fall, a blow to the head, or an electric shock, can cause a loss of memory for events in the recent past (Lindsay and Norman, 1972). This phenomenon is known as retrograde amnesia. More than 40 years ago C. P. Duncan (1949) demonstrated that retrograde amnesia could also be studied in an

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[1] *Portions of this work have been presented at the 13th International Conference of the IEEE Engineering in Medicine and Biology Society, Orlando, FL (Raslear, Akyel, Serafini, Bates, and Belt, 1991a).*

animal model. In his now classic experiment, rats learned a simple avoidance task and were subsequently given electroconvulsive shock. Those rats which received the shock 15 min after learning the task had poorer retention of the task when subsequently tested than did rats which received a similar shock 1 h after learning the task. It is now well established that a variety of treatments, including neurohormones and other drugs, are capable of modulating the retention of newly-acquired information in a variety of aversive and appetitively motivated tasks across a wide range of species (McGaugh, 1990). McGaugh (1990) indicates that changes in memory have been observed in bees, birds, fish, rodents and primates, and that memory can be either enhanced or diminished, depending on the particular treatment administered. As was the case with Duncan's classic experiment, the effect of any treatment is dependent on the time at which it is administered following the learning experience. Treatments which are administered 30 min or more following training have no effects on subsequent retention. Current theory is that memory consolidation is an active process with a time course of approximately 30 min. Memories which are not consolidated are lost.

A variety of hormones, including ACTH, vasopressin, substance P, cholecystokinin, and epinephrine, and opioid peptides have been found to exert a modulating influence on memory consolidation (McGaugh, 1990). It is usually the case in these studies that the memory modulating effect is not only time-dependent but also dose-dependent. For instance, low doses of epinephrine (3 to 100 µg/kg) enhanced retention of an inhibitory avoidance response, while a high dose (300 µg/kg) impaired retention. Any dose given 30 or more min after training was ineffective. Because of the biphasic influence of many of these memory modulating biochemicals, it is important to carefully design an experiment so as to detect changes in performance in either direction. Moreover, it is extremely important to note dose-response relationships since opposite effects can easily be obtained.

Since the modulation of memory by post-training experiences is common to both human and sub-human species, the use of animal models to study the effects of potentially toxic or harmful treatments on cognitive processes is highly attractive. The present experiment employs an animal model to study the effects of high power, short duration, pulsed microwave radiation on memory consolidation.

Although interest in the effects of microwave radiation on behavior dates to the 1950's, there is a dearth of creditable information on the effects of microwave radiation on cognitive

processes and a total absence of information regarding high power microwave radiation of the type which is of interest here. A recent review of the literature notes that "There are numerous reports - probably more than in any other area of claimed bioeffects of weak environmental EM fields - of subtle behavioral changes in situations lacking necessary rigor in experimental design, often defective in control procedures, with inadequate evaluation of intercurrent stimuli, and plagued by experimental data so noisy that even statistical evaluation does little to establish credibility for claimed interactions." (Adey, 1981, p. 481). As recently as 1984, a survey of the behavioral literature on microwave effects (Gage, 1984) did not report any animal studies of cognitive processes. The vast majority of animal studies have employed operant conditioning procedures and focused on changes in response rates on various schedules of reinforcement. Our own review of the microwave literature has uncovered two studies of relevance, although neither employed HPPM radiation.

Nealeigh, Garner, Morgan, Cross and Lambert (1971) exposed rats to a 2.45 GHz field at a power density of 50 mW/cm^2 for 20 min prior to learning the location of food in a Y-maze. The radiation was continuous wave (CW) and produced a 1-2 °C increase in body temperature. The rats were tested and exposed on three successive days. Both sham-exposed and microwave-exposed rats showed a reliable increase in correct responses as a function of training. Although there was no main effect of the microwave exposure, the experimental group performed better on the second and third days of training, as indicated by a reliable Days by Treatment interaction. More recently, Lai and his colleagues (Lai, Carino, Horita and Guy, 1989) studied the effect on rats of exposure to low-level pulsed microwaves (2450 Mhz, 2 μs pulses, 500 pps, SAR of 0.6 W/kg) for either 20 or 45 min prior to learning the location of food in a radial arm maze. Rats which received a 45 min exposure performed reliably worse than sham-exposed rats over a ten day training period. Rats which received a 20 min exposure performed reliably better than sham-exposed rats for the first two days of training. Thereafter there were no differences between the groups.

The biphasic influence of the microwave treatment in the Lai et al. (1989) study is reminiscent of the effects of numerous neurochemicals, such as opioid peptides, which McGaugh discusses in his review of memory modulation. Lai and his colleagues have, in fact, demonstrated that low-level microwave radiation probably activate endogenous opioids which in turn cause changes in cholinergic activity (Lai, 1987; Lai, 1988; Lai, Horita, Chou, and Guy, 1987a; Lai, Horita, Chou, and Guy, 1987b; Lai, Horita, Chou, and Guy, 1987c). The mechanism

by which endogenous opioids are activated is currently not known. Lai et al. (1987 and 1988) have shown that the biochemical reaction to microwave exposure is similar to that caused by various stressors, although the rats in their studies exhibit no obvious physical signs of distress during exposure. (It should be noted that Cordts et al. (1988) found that rats would not learn to avoid HPPM radiation, which further suggests that the animals are not physically distressed by even high power pulses of microwave radiation). It is possible, however, that the effect is direct. Recently Weaver and Astumian (1990) have developed a physical model which shows that very weak periodic electric fields can affect macromolecules in a cell's membrane. Such fields can couple to membrane-associated, enzyme-catalyzed reactions by means of an electroconformational mechanism. Since many membrane-bound enzymes have conformational transitions which make them sensitive to changes in the electric field that exists across the cell membrane, it is possible that electromagnetic fields can exert a direct effect on the production of hormones, neurotransmitters and other neurochemicals by the modulation of enzyme conformational transitions.

Given the lack of dosimetry in the Nealeigh et al. (1971) study, it is difficult to cite agreement with the performance enhancement found by Lai et al. (1989). Nevertheless, the two studies taken together suggest that memory function in animals which have been exposed to HPM warrant a closer examination. Either thermoelastic expansion of the brain, which is believed to be the cause of "microwave hearing" (Lin, 1980), or neurochemical changes in the brain could represent a sufficient insult to the brain to disrupt cognitive function and affect the performance of military personnel.

4.2 Task Description

The basic idea behind this experiment is that some learning takes place each time an animal or person experiences something new or significant. However, because new and/or significant events are constantly occurring to most living mammals, not all such learning is permanently preserved in memory. Rather, memory has two forms: a short-term form and a permanent form. Information which are stored in the short-term memory are volatile. If they are not moved into the long-term memory, they fade and cannot be retrieved. If the information are not made permanent, and an insult occurs to the brain (e.g., a blow to the head), the memory (information) will be lost.

A good example of short-term memory occurs when one calls information at a phone booth. If a pencil and paper is not available, the common strategy for remembering a new phone number is to sub-vocally repeat the number until it has been dialed. Within a few minutes after the call has been completed, most persons will have forgotten the number.

In the Y-maze task, we allow a thirsty rat to explore a novel environment until he discovers and drinks water for a few seconds. Because the rat is thirsty, the discovery and drinking of water makes this a significant event in his life (one that is likely to be remembered). Immediately following this learning, the rat is subjected to a treatment condition. If the treatment condition consists of being returned to its home cage, the short-term memory of the location of the water will be transformed into a permanent memory (memory consolidation). If the treatment consists of a trauma to the brain (e.g. microwave exposure), then memory consolidation will be disrupted.

4.3 Materials and Methods

4.3.1 Subjects

Thirty rats served as subjects. Ten rats were exposed to full power, 10 to -30 dB power, and 10 served as cage controls. The rats were water deprived prior to training and testing by restricting access to water to one hour each day. Microwave-exposed rats received 200 pulses from TEMPO, as described in the Introduction section.

4.3.2 Apparatus

The Y-maze consisted of three arms which were 23 cm long, 10 cm wide and 12 cm deep. The walls of the maze were made of Plexiglas and the floor was a grid constructed of steel bars. To facilitate observation of the subjects, the top of the maze was covered by a transparent Plexiglas sheet. Two of the arms were illuminated by identical 12 W lights and contained water bottles. A guillotine door was used at the starting arm to confine the subjects before the start of the experiment.

4.3.3 Behavioral Testing

Y-maze training was conducted following the procedures described by Sternberg et al. (1985). Briefly, five days prior to the experiment the rats were placed on a water deprivation schedule in which they are allowed 1 h access to water each day. On the day of exposure, each animal was placed into the starting arm of the Y-maze. One choice arm of the maze was illuminated (randomly selected for each rat), and a full water bottle was located at the end of that arm. The other choice arm was dark and an empty water bottle was located at the end of that arm. Each rat was allowed to explore the maze until the full water bottle was located and water was consumed. Errors were scored for entry into the dark arm, re-entry into starting arm, and exit of the lighted arm without consumption. Only one trial was allowed (finding and drinking from the water bottle) per rat. The available literature on this task indicates that for any treatment to affect memory consolidation, it must be administered within 30 min of training (McGaugh, 1990). Microwave exposures were begun within 1-2 min of learning the location of water.

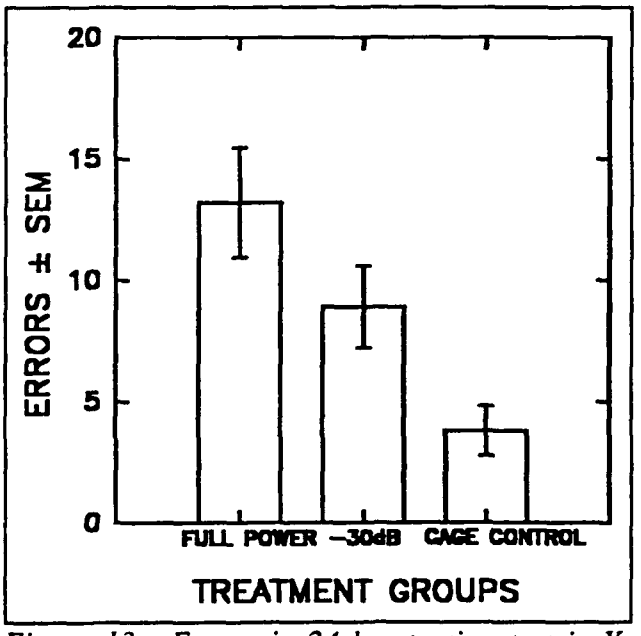

Figure 13. Errors in 24-h retention test in Y-maze for each treatment condition.

Y-maze testing occurred on the day following exposure (24-h retention test) using the same procedures. Errors, scored as in training, in locating the water were used to measure the retention of the original learning in the three groups.

4.4 Results

Errors on the 24-h memory consolidation test are shown in Figure 13. Exposed rats made an average of 13.2 errors, sham-exposed rats made an average of 8.9 errors, and the cage controls made an average of 3.8 errors. An Analysis of Variance on the error scores indicated a significant difference between the groups ($F_{2,29} = 7.31$, $p = .0029$). Post-hoc analyses indicated that the two exposed groups did not differ from each other, but were reliably different from the cage control group.

An analysis of the energy output for each of the 200 TEMPO pulses for each rat in the exposed and sham-exposed conditions was subsequently conducted. A statistically reliable correlation was found between the number of errors each rat made on the test day and the mean energy output of TEMPO on the previous day ($r = 0.3837$, $t_{18} = 1.763$, p [1-tailed] = .047). This is illustrated in Figure 14.

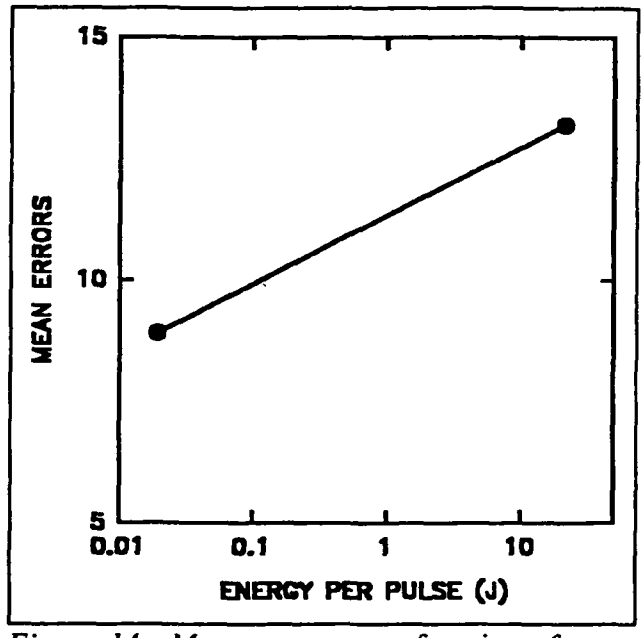

Figure 14. Mean errors as a function of mean energy (J) per pulse.

4.5 Discussion

Differences in errors between controls and the two exposure groups could be due a number of variables. Both exposure groups experienced additional handling stress, X-ray exposure, and noise, which were not experienced by the cage controls. The correlation between errors and mean energy output, however, strongly suggests that differences in the amount of RF energy experienced by the two exposure groups was critical. Reference to Table 3, moreover, indicates that noise exposure was highest for the -30 dB group, which is contrary to the positive correlation between energy output and errors. Similarly, Table 2 shows that X-ray exposure was also highest for the -30 dB group. The only variable which can account for the increase in errors is increased HPPM energy output.

It should be noted that the results of this experiment are consistent with the results of the bisection experiment. An increase in null responses, reflecting a decrease in decision confidence, could arise from a memory deficit. In other words, poor recall of the stimulus just presented, or difficultly in recalling the reference stimuli could result in an increase in decision time and null responses. Taken together, the bisection and memory consolidation experiments strongly suggest that HPPM may have a significant effect on memory function. This notion is strongly supported by the work which has been reported by Lai and his co-workers with low power microwave exposures, as noted above.

SECTION 5: TREADMILL RUNNING ENDURANCE [4]

Physical endurance is a very important performance characteristic for military operations. Although physical endurance can be measured in a variety of ways, we chose the treadmill because of its relevance to physiologjcal function and its easy cross-species comparability.

5.1 Background

The primary objective of this experiment was to examine the effects of high peak, low average power, pulsed microwaves on treadmill endurance in rats. As has been noted previously in this report, current civilian and DOD safety regulations specify an exposure limit averaged over a six minute period (see Bassen, 1989; D'Andrea, Cobb, and de Lorge, 1989) but do not limit peak power. The exposures of interest discussed here are of sufficiently short duration so as to not violate current safety regulations. However, the peak power levels are high enough to cause concern that they may have adverse effects, especially since there is a report that motor function in rats is affected by pulsed microwave exposure (Klauenberg, Merritt, and Erwin, 1988). This previous work, however, has not used the very high peak power levels which were used in this experiment. Moreover, since the treadmill endurance task has not been used to assess the effects of HPPM exposure, the present investigation, is exploratory and groundbreaking in several respects.

5.2 Task Description

Treadmill running is a standard laboratory procedure for the study of exercise physiology (Selkurt, 1971). However, apart from its more strictly physiological uses (e.g., the measurement of oxygen consumption [Bedford, Tipton, Wilson, Oppliger, and Gisolfi, 1979], muscle blood flow [Armstrong and Laughlin, 1985], fat metabolism [Shibata and Nagasaka, 1987], and hepatic function [Seelbach and Kris-Etherton, 1985]), the treadmill also provides a convenient means of assessing an animal's physical endurance following various drug and environmental manipulations (Fancesconi and Mager, 1979, 1980; Gerald, 1978; Hillegaart and Ahlenius, 1987), including ionizing radiation (Jones, Osborn, and Kimeldorf, 1967).

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[4] *Portions of this work have been presented at the First World Congress for Electricity and Magnetism in Biology and Medicine in Lake Buena Vista, FL (Akyel, Raslear, Hammer and Belt, 1992).*

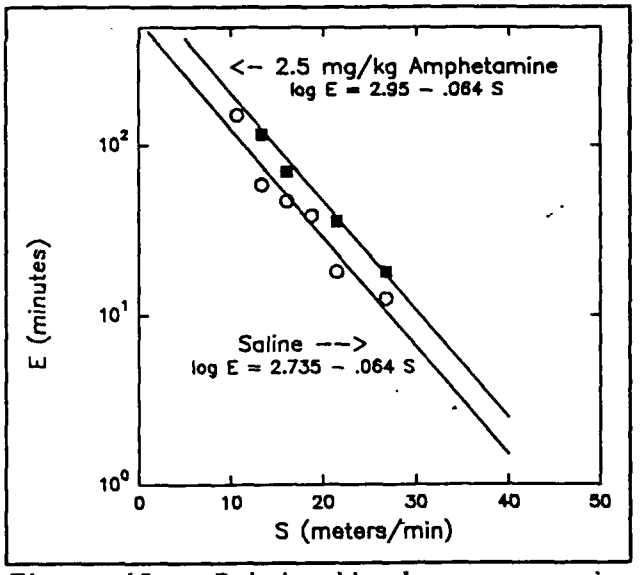

Figure 15. Relationship between running endurance and treadmill speed for saline and amphetamine. Data from Gerald, 1978.

The amount of time that an animal can run on a treadmill without faltering is a measure of the animal's endurance (E). Previous research has shown that an orderly relationship exists between E and the speed (S) at which the animal is required to run. Figure 15 illustrates this relationship with data from a study by Gerald (1978). Endurance is an exponential function of running speed (i.e., $E = 10^{\alpha-\beta S}$, where α and β are constants). Various treatments, such as drugs, produce increases or decreases in E which are reflected in changes in the constant α. This can also be seen in Fig. 15, which shows that E increases at all running speeds when animals are given 2.5 mg/kg amphetamine.

The relationship in Fig. 15 indicates that it is possible to select a running speed that will generate exhaustion within a specified period of time for the average rat. Groups of rats tested at a selected speed, but differing in their exposure to HPPM, can thus be conveniently compared with respect to their average endurance (time running).

In the present experiment, treadmill running at a selected speed was used to assess physical endurance immediately following exposure to HPPM. Literature searches conducted at WRAIR have indicated that this task has not been used to assess the effect of RF energy.

An animal model of physical endurance which is sensitive to changes in dose of RF radiation would be of great interest and use to the military medical community. Physical endurance is a key component of many basic soldier tasks, particularly in a combat environment. Radar transmitters and other generators produce brief high energy pulses of microwave energy which are part of the electromagnetic environment of Army and other military personnel.

5.3 Materials and Methods

5.3.1 Subjects

Twenty-six rats, similar to those previously described, served as subjects.

5.3.2 Apparatus

A commercially available small animal treadmill (Columbus Instruments, OH) with an adjustable incline and speed was used.

5.3.3 Behavioral Testing

For 8 days prior to exposure, rats received familiarization training on the treadmill apparatus for 5 min per day. Each rat was placed in the stationary treadmill at an 8° incline, and a relatively slow running speed (10 m/min) commenced. To motivate running, animals that left the moving treadmill and entered onto an adjoining shock grid received as many as 6 shocks of 200 ms duration at 163 V and 0.5 mA peak shock current (the lowest possible level on the equipment). Shocks could be avoided by running and could be escaped by re-entry onto the treadmill. After five minutes of training the treadmill was stopped, and the rat was returned to its home cage.

Following the training, 9 rats were exposed to 200 microwave pulses (80 ns width, 1 pulse/8 s, 3 GHz) using the TEMPO exposure system with 700 MW peak transmitted power (Bates and Bassen, 1989). Nine of the remaining animals were sham-exposed, and 8 rats served as cage controls. Sham- or microwave-exposed animals were restrained in a cylindrical plastic enclosure and positioned on an RF transparent platform inside a corner reflector. Exposures lasted approximately 25 min. Immediately following the sham- or microwave-exposure the animals were placed on the treadmill. Control animals were taken from their cages and placed on the treadmill. During the test session the speed of the belt was increased to 30 m per min with the same inclination. Running time was defined as the total time that each animal spent in the treadmill before receiving 3 consecutive electric shocks within 10 s without leaving the shock grid area.

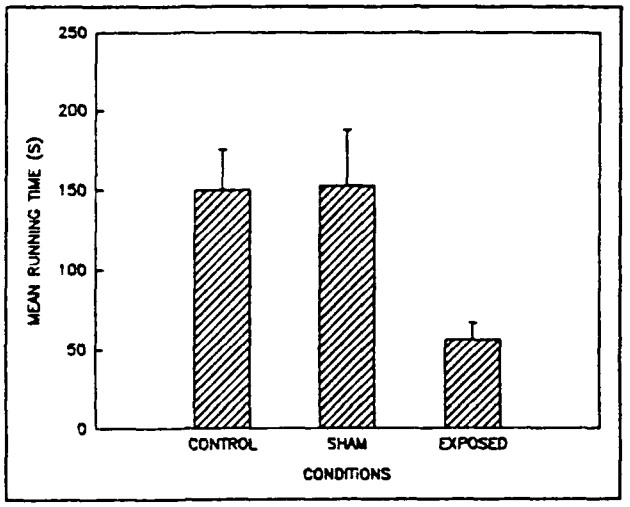

Figure 16. Mean running durations (± SEM) for microwave-exposed (full power), sham-exposed, and cage-control rats.

5.4 Results

The endurance of the animals was defined as the total running time of the subject during the high speed (30 m per min) test trial. As depicted in Figure 16, the mean (± SEM) running times of the cage control and sham-exposed animals were 150.13 s (± 25.65) and 152.89 s (± 35.18), respectively. On the other hand, microwave-exposed animals ran for 56.44 s (± 10.69), significantly less than the two groups which were not exposed to microwave fields. A Kruskal-Wallis one-way ANOVA test (χ^2 =6.02, $p < .05$) showed that reliable differences existed among the groups.

5.5 Discussion

The results indicated that exposure to 200 TEMPO pulses decreased the rats' endurance, as reflected in running time on the treadmill without faltering. While the current safety guidelines suggest that whole-body SAR should be limited to 0.4 W/kg and/or a local SAR of 8 W/kg, the TEMPO exposure system, which was used in the present experiment, produced a time-averaged midbrain SAR of 0.21 W/kg and time-averaged whole-body SAR of 0.07 W/kg. Thus, while exposure to microwave fields composed of very short pulses and extremely high peak power may be within present safety guidelines, the present data raise questions concerning the adequacy of these guidelines.

SECTION 6: MOTIVATION FOR FOOD (BEHAVIORAL ECONOMICS) AND CIRCADIAN RHYTHMICITY [1]

Because the term "motivation" is ubiquitous in psychology, it has various meanings in various contexts. In the present study, "motivation" means behavior which is directed by a physiological requirement for nutrients. The method which was chosen to study motivation was dictated by the recent success of behavioral economics to bring together the disparate fields of behavioral analysis and economics. The key to understanding work in behavioral economics is an understanding of a few basic economic concepts. All organisms, including people, consume commodities such as food. The amount of a commodity which will be consumed is a function of the price of that commodity. As the price increases, the consumption decreases. The relationship between price and the amount consumed (actually the slope of the function) is demand. The experiment reported here examined changes in the demand for food as a function of HPPM exposure. Since demand curves from classical economics (with humans as the consumers) are of the same form as demand curves from behavioral economics (with rats, pigeons and monkeys as the consumers), this aspect of the task should provide us with cross-species information on motivational effects of HPPM.

Another aspect of behavioral economic studies is that they often take place within a "closed economy". This means that the animals live in the experimental situation and earn all of the commodity they are working for within that situation. Thus, the method readily lends itself to a simultaneous examination of circadian rhythms.

6.1 Background

Many experimental treatments of military interest have multiple behavioral sequelae which currently require the use of several behavioral procedures and long periods of training to measure. This is also true of research on the behavioral effects of microwave radiation. The most reliable measures, to date, of microwave effects on behavior employ operant conditioning techniques (Gage, 1984). Typically these tasks require long periods of training with relatively few experimental subjects. As a result, it is difficult to establish functional relationships between the

[1] *Portions of this work have been presented at the 13th International Conference of the IEEE Engineering in Medicine and Biology Society, Orlando, FL (Raslear, Akyel, Serafini, Bates, and Belt, 1991b).*

physical parameters of microwave exposure (e.g., frequency, power density, specific absorption rate [SAR], etc.) and behavioral effects in a sufficiently large sample of animals. The inability to test more subjects in a shorter period of time is especially serious if the ultimate objective of the research is to establish exposure standards. In this case, a sufficient number of subjects is essential to establish and test the replicability of effects and to establish population estimates (i.e., means and confidence intervals) of the dose-response function(s).

A cost-effective method for the behavioral analysis of drugs, toxins, and other treatments of interest to the military community has recently been devised and tested (Raslear, Bauman, Hursh, Shurtleff, and Simmons, 1988; Hursh, Raslear, Shurtleff, Bauman and Simmons, 1988). The research reported here used this method to simultaneously assay microwave effects in two behavioral functions of military relevance (motivational and circadian rhythm function) within a seven day period for groups of six rats and required no training of subjects. The rapid testing method allows treatment effects to be replicated within seven days as often as required. A brief description of the testing method follows.

6.2 Task Description

6.2.1 The Behavioral Assay

Motivational Effects

Motivational effects are assessed using the experimental and analytic methods developed by Hursh (1984). In this method, subjects are required to perform a specific motor response (e.g., lever pressing) in order to obtain their entire daily ration of one or more commodities (e.g., food, water, etc.). Under these conditions, the consumption of a commodity, such as food, is partly determined by the price of the commodity (i.e., the number of responses required to receive a single food pellet, also known as the Fixed Ratio). A plot of the amount of a commodity consumed as a function of price yields a demand curve. The degree to which consumption declines with increases in price is called elasticity of demand. Elasticity directly determines whether a price increase will be accompanied by an increase in responding, no change or a decrease in responding. A demand curve with a shallow negative slope implies an increase in output or expenditure and is called inelastic demand. A demand curve with a steep negative slope implies a decrease in response rate and is called elastic demand. Hursh has found

that demand is a major dimension of motivation which can change along two different dimensions: intensity and slope. Recent experiments have indicated that these two dimensions of demand are sensitive to such variables as the nature of the commodity, the availability of substitutes, the level of deprivation and pharmacological treatments.

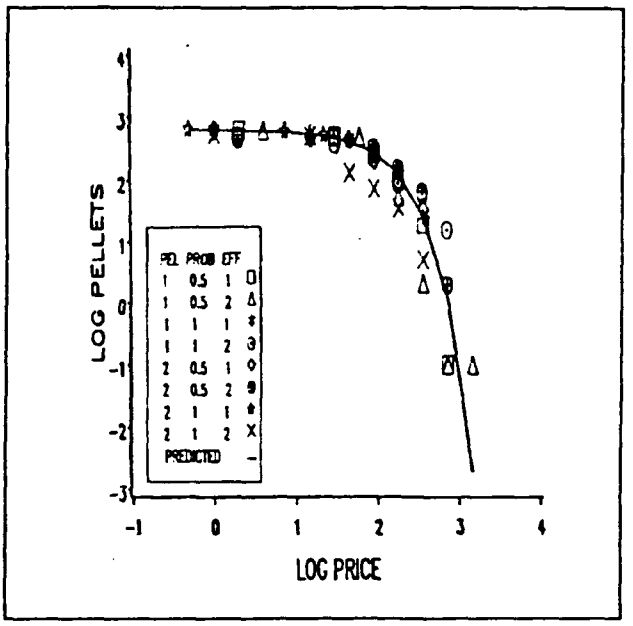

Figure 17. Log pellets earned as a function of log price. See text for details.

Whereas the basic experiments on demand analysis required long periods of time to complete (e.g., approximately 30 days to determine a single demand curve) the present method compresses the test period into 7 days. This is accomplished by tripling the response requirement each day (i.e., a 300% daily increase). Previous work had increased the response requirement by much smaller daily increments (e.g., 20%), and so required longer to produce a complete demand curve. Extensive testing of this method has now been completed. For instance, Figure 17 presents median demand curves for food as a function of price with response effort (W), number of pellets per Fixed Ratio (M), and the probability (α) of receiving pellets (per Fixed Ratio) as parameters (Hursh et al., 1988). These three factors, together with the response requirement (N, fixed ratio) determine the unit price (P) for food:

$$P = \frac{NW}{\alpha M}.$$

Predicted food consumption as a function of price can be determined from:

$$Q = L\, P^{b}\, e^{-aP},$$

where Q is the number of pellets consumed, P is the unit price, L is the level of consumption at a unit price of 1.0, e is the number 2.718..., and a and b are constants. The solid line in Figure 17 is the predicted food consumption for the experimental conditions. At each required number of lever presses, the rats would receive either one or two pellets of food with a probability of 1.0

or 0.5 and with 1 or 2 units of effort (weight of the lever which the rat presses). As can be seen, the method provides data which are consistent with the theoretical framework of behavioral economics.

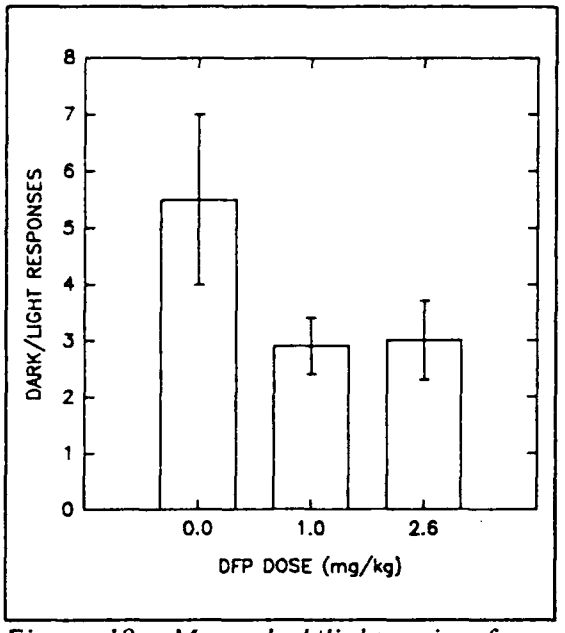

Figure 18. Mean dark/light ratio of rats treated with various doses of DFP, an anticholinesterase drug.

Circadian Effects

Since the rats receive all of their food in the experimental cages, data are naturally collected over a 24 hour period. Thus, it is a simple matter to record the data according to the time of day. Previous research (Raslear and Kaufman, 1983) has shown that diisopropyl phosphorofluoridate (DFP, an anticholinesterase agent) produces a long-term disruption of circadian patterns of behavior, and other treatments may produce a similar change. Because it is a major biological system, information that a treatment disturbs the normal circadian pattern is important, and the military relevance of such a change is apparent. Figure 18 presents the kind of data which will be used to index the function of the circadian system (from Raslear, Leu and Simmons, 1986). The figure shows the Dark-Light Ratio (number of responses produced in the dark divided by the number of responses produced in the light) for groups of rats given 0, 1.0 or 2.6 mg/kg DFP. Note that both high and low doses of DFP produced changes in the normal circadian distribution of responding.

6.3 Methods and Materials

6.3.1 Subjects

Twenty four male Sprague-Dawley rats were used as subjects.

6.3.2 Apparatus

Behavioral measurements were conducted in six modified hanging wire cages (Wahmann Co.) situated in an environmental chamber. Each cage was 24.1 cm long, 20.3 cm wide, and 18.4 cm deep. A response lever, water bottle, and food magazine were mounted on the front of each cage. The response lever was 5.1 cm long and 1.3 cm wide and extended 1.3 cm into the cage. The lever required 0.265 N to operate. The lever and food magazine were mounted 5.1 cm above the floor, on the center of the cage front. The water bottle spout extended into the cage at the level of the magazine and lever on the same side of the cage as the magazine. Food pellet dispensers (Gerbrands, Model G5100) were mounted above each cage. These delivered 45-mg food pellets (BioServ) according to the schedule requirements described below.

6.3.3 Behavioral Testing

One week prior to the experiment, rats were adapted to the laboratory conditions and the light/dark cycle by being housed in ordinary hanging wire home cages within the environmental chamber where the experimental cages are housed. Following this adaptation period, all rats were exposed to microwave radiation in accordance with their group assignment.

The rats were exposed to one of three conditions. "Full power" rats were exposed to 700 MW peak output power (400 pulses, 80 ns width , 1 pulse/5 sec), "-30 dB" rats were exposed to 700 kW peak output power, and "control" rats were placed in the exposure chamber for a similar period of time as the other two groups, without operating TEMPO. Following exposure, each rat was placed in one of six similar home cages which was equipped with a response lever and food pellet dispenser. On the first day in the test apparatus a single response produced a single 45-mg food pellet (BioServ). The animals received no prior lever-press training and were not food-deprived. Data from the first day were not used to allow for individual differences in lever-press acquisition. On the second day the response requirement remained at 1 response/food pellet (FR 1, Fixed Ratio 1). On subsequent days the response requirement was increased to 15, 45, 90, 180 and 360 responses/pellet. The animals were free to respond at all times of day. A PDP 11/73 computer recorded responses and food pellet deliveries, and controlled the sequence of response requirements. A 12:12 light:dark cycle (lights on at 0600, off at 1800 hours) was in effect in the chamber where the test cages were located. Water was available at all times, but the only source of food was what the rats earned in the experiment.

6.4 Results

Figure 19 (left panel) shows the mean number of pellets consumed by each group as a function of the price of the food. The pattern of decreasing consumption as price increased is typical of rats (and even people when the price of an essential commodity is increased) in this situation (Raslear et al., 1988; Hursh et al., 1988) . There are no apparent differences between groups, and it must be concluded that HPPM has no effect on food motivation.

Circadian function was assessed by comparing the number of food pellets obtained during the day and the night. Rats are nocturnal and normally consume 90 - 100% of their food during the night. Deviations from this pattern of feeding are rare.

With the exception of FR 1, there did not appear to be an effect of HPPM on circadian function. Figure 19 (right panel) shows the FR 1 data. The two exposure groups have a clearly different pattern of circadian food intake relative to the controls. A Kruskal-Wallis test on the FR 1 data indicated that a reliable difference exists between the groups ($p < .05$). Since total

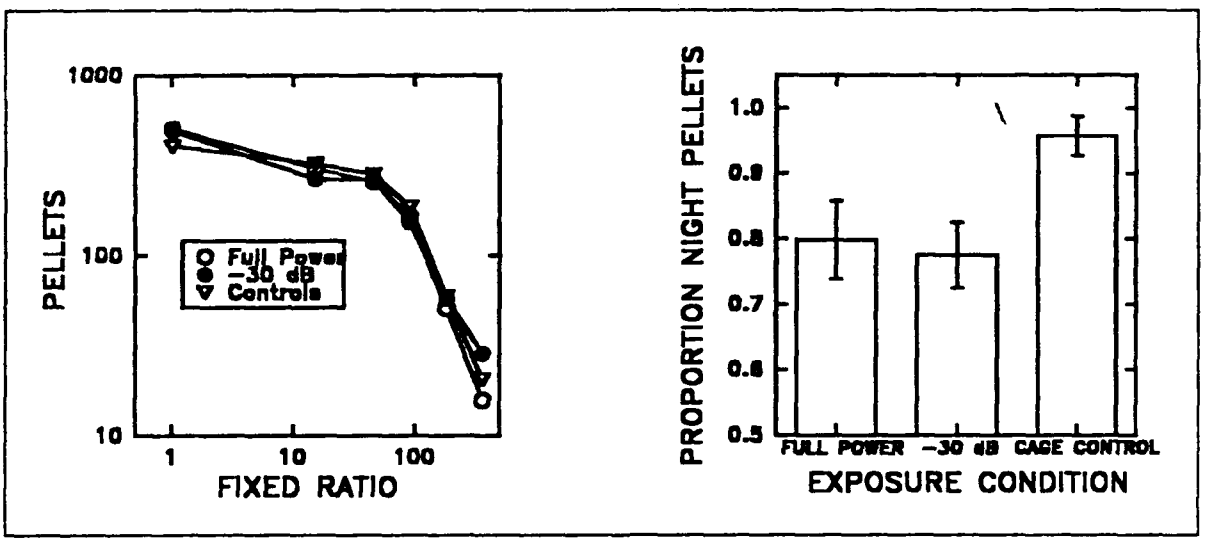

Figure 19. Left Panel: Mean pellets consumed as a function of cost (fixed ratio) and exposure condition; Right Panel: Mean proportion of pellets consumed during the dark cycle.

consumption at FR 1 was equivalent for these groups (see Figure 19, left panel), the exposed animals apparently consumed more food during the day.

6.5 Discussion

HPPM does not appear to affect food motivation in the rat. Previous research with this method (Raslear, Shurtleff, Rigamonti, Hursh, and Simmons, 1990) has shown that rats with lesions in the ventromedial hypothalamus (VMH) exhibit a markedly different pattern of consumption as the price of food is increased. The amount of brain damage in the VMH which is necessary to produce a measurable effect with this method, however, is not currently known. Thus, there may be damage to the VMH which cannot be detected.

The data, however, suggest that the circadian pattern of consumption may be altered. As noted previously, rats rarely depart from a circadian pattern of consumption in which 90-100% of their daily food intake is consumed in the dark. Disrupted rest-activity cycles are a significant human medical problem, so further investigation of this effect is clearly warranted.

SECTION 7: PORSOLT SWIM TEST (BEHAVIORAL DESPAIR)

Affect or mood is a very important constituent of human existence, but it is only rarely addressed in experimental studies with animals because of the lack of suitable models. Recently, however, several models of depression or despair have been developed for use in the pharmacology community to aid in the rapid screening of drugs for antidepressant activity. The following experiment utilizes one of these models to explore the effects of HPPM on affect in the rat.

7.1 Background

Most of the reported effects of radiofrequency electromagnetic (RFEM) fields on human beings have come from Eastern Europe (NCRP, 1986). A typical set of symptoms identified as the neurasthenic syndrome or "microwave sickness" is a variable collection of complaints that includes irritability, headache, lethargy, insomnia, impotence, mnemonic disorders, and loss of libido (Sadcikova, 1974). When persistent, this set of symptoms is like that labeled the "chronic depressive reaction" in the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1987).

Few studies of the human response to RFEM irradiation have been reported in the Western literature. Although they were not limited to behavioral effects, individual cases that had attracted public attention were not investigated scientifically. A classified experimental study of possible behavioral and physiological effects of RFEM radiation in primates called the Pandora Project and a study named Big Boy, which was initiated in late 60s and early 70s to investigate psychological and physical effects in humans, yielded inconclusive results. One study, that involved American employees of the United States Embassy in Moscow, did reveal considerable neurasthenic symptomology, but these symptoms were not correlated with levels of exposure (Lilienfeld, Tonascia, Tonascia, Libauer, Cauthen, Markowitz, and Weida, 1978).

One of the weak points of these projects was the inability to measure correct dose (exposure) levels in the subjects. However, in recent years, dosimetric procedures have become a very important and indispensable part of RFEM research. The techniques and the equipment used to determine the dose levels absorbed by the subjects have dramatically improved (Mathur, Akyel, and Lu, 1992).

On the other hand, the major problem in studying depression in the laboratory setting was the absence of an animal model (Willner, 1984). In the last 10 years a model developed by Porsolt, LePichon, and Jalfre (1977) has raised a considerable amount of interest and has been used extensively in testing antidepressant substances. Much information regarding this test is now available. The antidepressant procedures useful in humans produce an effect in this test and there is a significant correlation between clinical potency and potency of antidepressants in the behavioral despair test (Alonso, Castellano, Afonso, and Rodriguez, 1991).

7.2 Task Description.

In Porsolt's behavioral despair test, rats or mice are forced to swim in a confined inescapable cylinder containing water for 15 min. After an initial period of vigorous activity, the animal becomes immobile. On the subsequent immersion (24 hours later), the onset of immobility is much more rapid. These behavioral observations suggest that the animals, on finding that escape is impossible, give up trying and resign themselves to the experimental conditions. Porsolt, Anton, Blavet, and Jalfre (1978) hypothesized that the observed immobility reflected a state of lowered mood or hopelessness in the rat and predicted that immobility would be reduced by treatments which are known to be affective in alleviating depression in humans. Thus, tricyclic antidepressants (Kitada, Miyauchi, Satoh, and Satoh, 1981), monoamineoxidase inhibitors (Drago, Continella, Mason, Hernandez, and Scapagnini, 1985), as well as electroconvulsive shock (Kawashima, Araki, Uchiyama, and Aihara, 1987), and REM sleep deprivation (Hodgson, 1984) tend to reduce immobility in forced-swimming test.

The extent to which the forced-swimming test is specific for depression has been questioned, and it has been suggested that it only measures general activity which can easily be measured by an open-field activity monitor (Borsini, Volterra, and Meli, 1986; Nishimura, Tsuda, Oguchi, Ida, and Tanaka, 1988). However, Alonso, Arevalo, Afonso, and Rodriguez (1991) showed that open-field activity and forced-swimming tests are modified in a different way by drugs or by electric shock. They also found no significant correlation between activity in the open-field test and in the forced-swimming test.

In recent years a thorough exploration of the procedures used during the forced-swimming test revealed that trained observers are as good as an automated version in recording immobility time and that the swimming performance is sensitive to water depth (De Pablo, Parra, Segovia,

and Guillamon, 1989). Moreover, it has been found that swimming in a cylinder in which another rat had been swimming decreased the immobility time due to a pheromone released by rats after a 3.5-min or longer swimming period (Abel and Bilitzke, 1990; Abel, 1991).

Given the recent improvements in dosimetry for high-power microwave studies, and a better understanding of the validity of the forced-swimming test paradigm, it would seem that this was an opportune time to study neurasthenic sympthomology of "microwave sickness" using the forced-swimming test as an animal model of depression. The objective of this experiment was to determine the effects of high energy pulsed microwave exposures on forced-swimming test in rats' performance, which is a widely accepted and reliable model for human depression and behavioral despair.

7.3 Materials and Methods

7.3.1 Subjects

Male albino Sprague-Dawley rats were housed in temperature and humidity controlled modules designed for long term housing in the DMR laboratories. The vivarium was maintained at 22° C, 50% relative humidity, under a 12 hour light-dark cycle; lights activated at 0600.

7.3.2 Apparatus

All exposures were performed with pulsed microwave radiation at DMR laboratories using a 700 MegaWatt peak power pulsed microwave generator (TEMPO) operating at 3 GHz. TEMPO pulses are 80 nsec width and be produced at a rate of 1 pulse/8 s. A corner reflector was used to concentrate radiation from a transmitting antenna onto a platform immediately inside the reflector. This increased the exposure density by a factor of 10. The animals to be exposed were restrained in plastic holders on the platform. The platform was located in the reflector and in the center of the beam from the transmitter's horizontally polarized slot antenna.

For Porsolt's forced-swimming task, a clear vertical Polycarbonate cylinder (height 50 cm; diameter 20 cm) containing 22 cm of water maintained at the room temperature (23° C) was used.

7.3.3 Behavioral Testing

Rats were plunged individually into the cylinder containing water. The water level was adjusted such that as the rat floated with its nose above the water and its rear paws could not touch the bottom of the container. After 15 min in the cylinder the animals were removed and allowed to dry for 30 min in a heated enclosure (28° C) before being returned to their individual cages. One day later the rats were again placed in the cylinder and the duration of immobilization was quantified during the following 5 min. Because of the alarm substance (low volatile pheromone) produced by the rats during the swimming (Abel, 1991), the cylinder was cleaned and the water was replaced after each task.

A rat was judged to be immobile when it was floating passively, making only those movements necessary to keep its head above the water. The swimming behavior was tape recorded via a video camera and the cumulative time spent immobile was recorded by stopwatch by an individual who was unaware of the prior treatment of the animals.

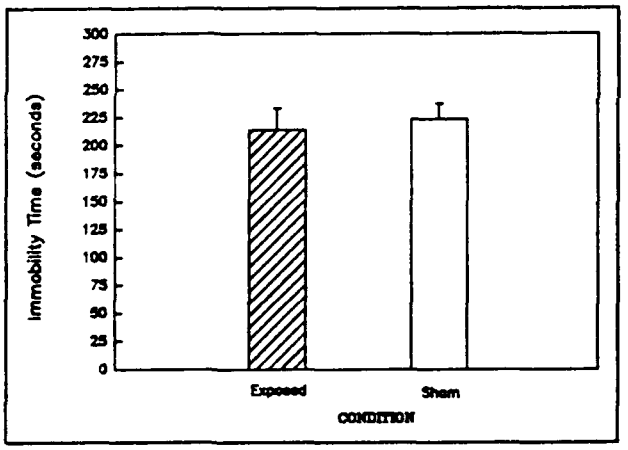

Figure 20. Immobility durations for exposed and sham animals in the Porsolt Swim Test.

Prior to the second immersion, animals were either exposed to 200 full power TEMPO pulses that lasted 30 min (exposure group) or sham-exposed for the same period of time in the same microwave chamber. Each group consisted of 13 animals.

7.4 Results

In this study immobility is considered to be an indicator of despair. It was hypothesized that there would be a difference in the duration of immobility time between the TEMPO exposed group and the sham group. As can be seen in Figure 20 an independent t-test comparing the two groups showed that the nonexposed group stayed immobile in the water slightly longer than the exposed group but this difference was not reliable ($t = .41$, $df = 24$, $p = .687$).

As an additional measure of stress, rat droppings were counted in all the sessions. However, an independent t-test did not reveal any reliable difference between the number of droppings of the sham and the exposed groups ($t = .82$, $df = 24$, $p = .421$).

7.5 Discussion

The present experiment used Porsolt's forced-swimming task as a model of depression to investigate the effects of acute exposure to high power pulsed microwave fields. Evidence that HPPM affected performance was not found in this study. Hence, we cannot conclude that HPPM affects depression or despair. However, this result might be due to the fact that only acute RF exposures as opposed to chronic exposures were used. According to the literature, symptoms of neurasthenic syndrome or "microwave sickness" develop after a long-term exposure to microwave fields. Since there are indications that chronic exposures may yield quite different results it is important that this area be pursued further. Future experiments on high power microwaves need to focus more on the effects of chronic exposures to such fields.

SECTION 8: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

8.1 Summary of Results

The TEMPO exposure system is a unique experimental platform for the study of the biological and behavioral effects of radiofrequency electromagnetic radiation. The system is capable of producing very high peak-power outputs (approximately 700 MW) for very short periods of time (approximately 80 ns). As a result, the average power is well within the range in which no heating effects should be observed (approximately 2 W). The system is capable of exposing animals within an anechoic chamber which is temperature- and humidity-controlled, so that good environmental control can be arranged during the conduct of experiments.

Calorimetry and thermometry indicate that the time-averaged SAR produced by exposure to TEMPO is well-below the recommended exposure limits. The peak SAR (associated with the peak power output of 700 MW) is 25.2 MW/kg, but the time-averaged SAR is merely 0.072 W/kg. Local SARs are in substantial agreement with this result, as can be seen in Figure 2. The highest SARs (0.552 W/kg) were located subcutaneously in the region of the nose, but were still within an order of magnitude of the time-averaged SAR obtained with calorimetry. The average SAR in Figure 2 (based on the 24 locations) is 0.17 W/kg, a value which is in substantial agreement with the time-averaged SAR.

Five different tasks were used to determine if exposure to high-peak, low average, power microwave pulses has an effect on behavior in the intact animal. Each task addresses different functions within the behaving organism, and some address similar functions. The temporal bisection task provides measures of sensitivity to perceived differences in time, as well as allowing inferences concerning such cognitive functions as memory, attention, and decision-making. The Y-Maze task was used to determine if HPPM can disrupt the consolidation of a short-term memory into a permanent memory. This task addresses the issue of memory function very directly, so that the information it provides is not necessarily redundant with that obtained from the bisection task. The treadmill task, on the other hand, is mainly concerned with physical endurance and has little functional overlap with any of the other tasks except the behavioral economics task. Motivation for food, as indicated by the willingness of animals to press a lever repeatedly for food, may be construed as similar to running on a treadmill to avoid foot shock. A primary difference, however, is that running on a treadmill is known to affect the concentration

of oxygen and carbon dioxide in the blood, whereas lever-pressing probably does not. Thus these tasks differ with regard to physical endurance while bearing equally on the question of motivation. The Porsolt Swim Test, because of its documented ability to predict the anti-depressant qualities of drugs, is probably best considered to be a functional test of mood (in this case despair or depression).

The bisection task indicated that time perception (i.e., the relationship between physical duration and perceived duration, as indicated by changes in the bisection point) is probably not affected by HPPM. Sensitivity to changes in time (i.e., the ability to detect differences in duration, as indicated by the slope of the psychometric function and A'), however, may be affected. To the non-expert in psychophysics this may sound contradictory, but it is not. The task is designed such that estimates of the bisection point are not based on the same information that discriminability estimates are. The bisection point reflects only changes in the mean rate of the clock, but discriminability uses the mean rate and its variance. Thus, this result suggests that a change has occurred in the variance of the timing system of the rat. The result that both session time and null responses are also affected by HPPM further clarifies the situation. Null responses are clearly under stimulus control (i.e., they are a function of the stimulus duration presented), so it is impossible to claim that this effect is a simple work stoppage (in which case the stimulus duration would be irrelevant). Null responses reflect decision-making, and decision-making is dependent (see Figure 4) on the output of the memory function. Memory function contributes to the variance of the clock rate. Therefore, it seems likely that the effects of HPPM on null responses and on time discrimination are both due to a change in memory function.

As noted above, memory function is directly assessed in the Y-maze memory consolidation task. If memory function is affected by HPPM in the bisection task, then performance in the Y-maze task should also be affected by TEMPO exposures. Such an effect was, in fact, observed, thereby lending strong support to the hypothesis that HPPM affects memory function.

Unlike the bisection and Y-maze tasks, the treadmill task is primarily a test of physical endurance. Treadmill running is widely used to study exercise physiology and the factors which affect fatigue. The treadmill study clearly demonstrated that exposure to HPPM affects the amount of time that animals can run on a treadmill. We interpret this result to indicate that the rats which are exposed to HPPM have less physical endurance than control animals. It is also

possible, however, that HPPM affected the motivation of the exposed rats (i.e., foot shock seemed less painful or salient), or that HPPM caused the rats to despair (i.e., "I might as well give-up running now, since shock is inevitable"). These interpretations were addressed in the studies on motivation and behavioral despair.

The behavioral economics task has demonstrated sensitivity to motivational variables. For instance, animals with brain lesions in the VMH exhibit an altered pattern of food intake in this task. Since HPPM-exposed rats did not show an appropriately altered pattern of food intake relative to controls in this task, it appears unlikely that motivation is affected by HPPM. This supports our contention that physical endurance was affected by HPPM in the treadmill task. It should be noted that the change in circadian rhythm of eating which was produced by HPPM in the behavioral economics task is not inconsistent with a change in physical endurance. Numerous human studies have demonstrated that the performance of physical tasks is highly dependent on the time of day (Brown and Graeber, 1982). Thus, it is possible that the decrease in physical endurance observed in the treadmill task is a consequence of altered circadian rhythms.

The Porsolt Swim Test, on the other hand, is a standard test for anti-depressant activity in drugs. As such, we consider it to be an indicant of "mood" in the rats. In particular, it provides information concerning depression or despair. Thus, the finding that animals exposed to HPPM do not differ from control animals suggests that HPPM does not affect mood. Performance decrements on the treadmill following HPPM treatment, therefore, are probably not due to depression or despair.

We hypothesize, therefore, that HPPM has at least two adverse effects in rats. First, as demonstrated in the temporal bisection and Y-maze tasks, HPPM appears to interfere with memory. This effect occurs at the level of memory consolidation (Y-maze) task, and may also involve other memory processes such as comparison of short- and long-term memory traces (bisection task). This conclusion is supported by the work of Lai and his colleagues (Lai et al., 1989) who have also found evidence that microwave exposure affects memory and learning. Second, HPPM reduces physical endurance (treadmill). The mechanism of this effect is possibly due to disrupted circadian rhythms (behavioral economics task), and is probably not due to changes in motivational (behavioral economics task) or mood states (Porsolt swim test). The mechanisms of both effects require additional research and investigation. This would be best done with tasks which specifically target the functions of interest and/or alternative mechanisms.

8.2 Safety Standards

As noted in the Introduction, current safety standards do not address the issue of the peak power that can be used to achieve a "safe" microwave dose. In the experiments reported here, time-averaged SARs were all well below the suggested whole-body dose of 0.4 W/kg. This suggests that peak power may also have to be considered in the formulation of maximum permissible exposure levels for HPPM.

SECTION 9: FUTURE DIRECTIONS FOR RESEARCH

The TEMPO is a unique, highly reliable HPPM source which has allowed replicable investigation of the implications of low average, high peak power microwave exposures. Since the acute exposures which have been pursued to date appear not to produce any long-term or cumulative effects (see particularly the temporal bisection experiment), it is important to redirect research objectives toward developing protocols involving long duration exposures with extended post exposure evaluation of subjects. This approach is especially important since it would more nearly approximate the occupational hazards prevalent within the Department of Defense.

Historically, the military services often find themselves subjected to public, scientific and legal expressions of concern regarding soldier safety following introduction of new systems. Many weapon systems currently under development or in the inventory take advantage of highly sophisticated technologies, such as radars, which emit radiofrequency (RF) radiation. Additionally, to reduce or minimize the potential for detection, these same systems employ short burst, high energy emissions that could potentially present a hazard to soldiers over the long term. Current safety standards do not appreciably consider these potential hazards since they are currently not clearly defined or understood. Therefore, it is imperative that safety thresholds be determined in a scientifically defensible manner.

To date, effects have been studied within a short post-exposure time frame. Most of our behavioral measurements have occurred within 24 h of exposure to HPPM. Virtually nothing is known concerning the effects of chronic HPPM exposures of the type produced by TEMPO. There is a clear need to determine if there are effects of HPPM during exposure and at longer post-exposure test intervals. The presence of acute exposure effects enhances the importance of extending our research in this direction. We suggest that several areas be addressed in future research on this basis.

Although noise and ionizing radiation do not appear to correlate with changes in behavior that are RF-dose dependent, it would be desirable to expose animals to HPPM in the absence of such environmental disturbances. The need to eliminate extraneous stimulation from the exposure environment and the need to study chronic exposures both indicate a requirement for additional research on bioeffects exposure platforms.

We have suggested that the high peak-power produced by TEMPO may be responsible for the observed behavioral effects. In the near future we plan to compare TEMPO exposures with comparable average power CW source exposures. Acute and chronic exposures should also be compared in this regard. In-field behavioral tests are particularly challenging to perform, but may provide the best indicant of possible adverse effects.

To date, very few behavioral paradigms have been examined. The logical approach is to extend investigation into those areas where effects have already been discovered. This however, should not preclude other exploratory investigations with untested behavioral paradigms.

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